June 2021 | EN

flocktype® AIV Ab

Validation Report

For detection of antibodies to Avian Influenza A Virus

INDICAL BIOSCIENCE

Contents

1 Introduction

1.1 Intended use

The flocktype AIV Ab is a specific and sensitive ELISA for detecting antibodies to Avian Influenza A virus in serum and plasma samples from chicken and turkey.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 435) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

1.2 General information

The flocktype AIV Ab is a highly sensitive and specific solution for the detection of antibodies to Avian Influenza A Virus (AIV) in avian serum and plasma samples.

Avian influenza is caused by various strains of Influenza A Virus. It infects wild fowl as well as domestic poultry. Influenza A Virus strains are classified as low pathogenic or highly pathogenic. Highly pathogenic strains belong to subtypes H5 or H7 and can cause the severe systemic symptoms known as bird flu or avian flu. The flocktype AIV Ab uses a structural protein of AI virus prepared by recombinant technology as antigen. This protein is highly conserved amongst AIV strains and strongly immunogenic. Thus all subtypes of Influenza A viruses will be detected.

With the flocktype AIV Ab, antibody titers can be determined in the chicken/ turkey.

1.3 Description of the test principle

The microtiter test plate is coated with a recombinant structural protein from the virus. During sample incubation AIV-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing.

The anti-IgY-HRP conjugate detects serum antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. In the presence of AIV-specific antibodies, within the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution.

The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of anti-AIV antibodies in the sample.

1.4 Kit contents

flocktype Salmonella Ab	**(2)**
Cat. no.	**FT274012**
Number of plates	**2**
Test Plate: microtiter plate with 96 wells, coated with non-infectious AIV antigen	2
Sample Diluent, ready to use	1 x 125 ml
Negative Control, ready to use	1 x 3.5 ml
Positive Control, ready to use	1 x 3.5 ml
Wash Buffer, 10x concentrate	1 x 125 ml
Conjugate, ready to use	1 x 24 ml
TMB Substrate, ready to use	1 x 24 ml
Stop Solution, ready to use	1 x 24 ml
Handbook	1

1.5 Storage

The components of the flocktype AIV Ab ELISA should be stored at 2-8°C and are stable until the expiration date stated on the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18-25°C) to avoid salt crystallization. Store the remaining test strips in the re-sealed foil pouch with desiccant at 2-8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

1.6 Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

2 Procedure

2.1 Things to do before starting

Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Sample Diluent and Wash Buffer (10x), dissolve by gentle swirling and warming.

Wash Buffer: Dilute Wash Buffer (10x) 1:10 in distilled water, e.g., for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Serum/plasma samples: Prior to sample analysis, with serum/plasma samples, dilute 1:500 in Sample Diluent (e.g., dilute 1 µl sample in 499 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Change pipet tips for each sample.

Alternatively, serum/ plasma samples can be diluted from a pre-dilution (1:50 in Sample Diluent) directly in the Test Plate (see Procedure step 1a).

Controls: Controls are ready to use and do not require dilution.

2.2 Protocol

1. Pipet 100 µl of each of the ready to use Negative Control (in duplicates) and Positive Control (in duplicates) and the 1:500 samples into the Test Plate wells.

1a. Alternatively, pipet 90 µl of Sample Diluent in each well and add 10 µl of the 1:50 pre-diluted sample. Mix well.

Note: Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

2. Incubate for 30 min at room temperature (18-25°C).
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
5. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).
6. Remove solution from wells by aspiration or tapping.
7. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.
10. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
11. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

3 Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.7.
- The mean value (MV) of the measured OD value for the Negative Control (NC) must be ≤ 0.2.

In case of invalid assays, the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Endpoint titers are calculated from the S/P ratio at a 1:500 dilution using the following equation:

$$Log_{10}\ Titer = 1.54\ (Log_{10}\ S/P) + 3.77$$

Data interpretation of the results

- **Samples with S/P-ratio < 0.3 are negative**.
  Specific antibodies to AIV could not be detected.

- **Samples with S/P-ratio ≥ 0.3 are positive**.
  Specific antibodies to AIV were detected.

4 Characteristics of the test

4.1 Diagnostic sensitivity

Procedure

Sera from infection experiments performed at the German National Reference Laboratory (NRL) for Avian Influenza (H5, H7; 6, 19, 21 days post infection) and from private sources (H1, H3, H6, H7, H9) were tested with the flocktype AIV Ab ELISA.

Results

Test results are shown in Table 1. All *n* = 38 sera from infection experiments were tested positive in the flocktype AIV Ab ELISA. The diagnostic sensitivity is therefore 100 %.

Table 1. Diagnostic sensitivity for *n* = 38 serum samples from infection experiments tested with the flocktype AIV Ab

Sample set	**Origin**	***n***	**flocktype AIV Ab**			
			pos	**neg**	**Mean value S/P ± SD**	**Diagnostic sensitivity**
Set 1	NRL	33	33	0	-	100 %
Set 2	private	5	5	0	-	100 %
total		**38**	**38**	**0**	**1.18 ± 0.59**	**100 %**

n = amount of samples; SD = standard deviation
Results: pos = positive, susp = suspect, neg = negative

Conclusion

The flocktype AIV Ab shows is a reliable tool to detect AIV-specific antibodies in chicken and turkey samples.

4.2 Specificity

Definition

Specificity measures the proportion of true negatives (non-infected animals) correctly identified by the new test. Non-infected and non-vaccinated reference animals giving positive results are rated false positive.

4.2.1 Specificity for sera from SPF or AIV-antibody negative control animals

Procedure

Sera from uninfected chickens, vaccinated with the recombinant vaccine ILTV-H7 (see 4.4 H7N1 vaccination and challenge study; 15 days post vaccination) were obtained from the German NRL (*n* = 8). The recombinant vaccine ILTV-H7 is an Infectious Laryngotracheitis Virus with an insertion of the AIV hemagglutinin H7 gene from AIV A7/chicken/Italy/445/99 (H7N1) leading to the production of AIV hemagglutinin H7-specific antibodies. Hemagglutinin H7 is not the coating antigen used for the flocktype AIV Ab.

In addition, *n* = 184 sera from SPF chickens were tested with the flocktype AIV Ab.

Results

All *n* = 192 sera correctly scored negative in the flocktype AIV Ab (Figure 1). The mean value of the S/P-ratios is 0.005 ± 0.021. With this, the mean value of the S/P-ratios is 60-times from the cut-off (S/P = 0.3). Diagnostic specificity is 100 %.

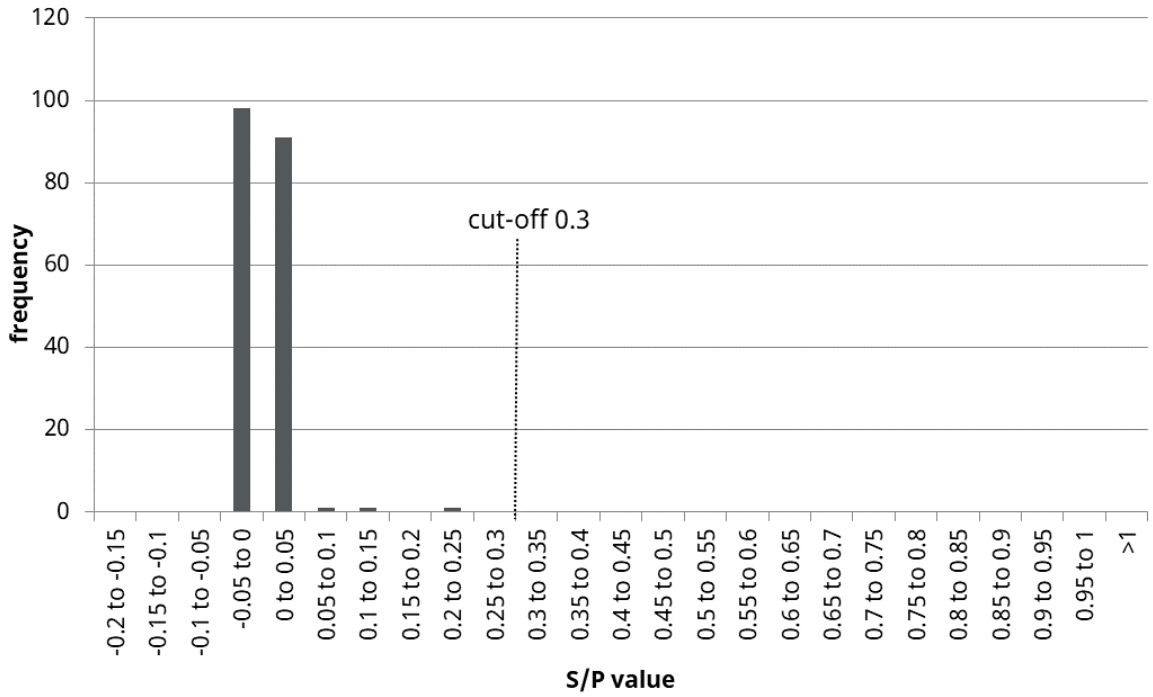

Figure 1. Frequency distribution of the S/P values of *n* = 192 AIV-antibody negative samples from SPF animals or control animals in a vaccination trial, tested with the flocktype AIV Ab

Conclusion

These results indicate an excellent diagnostic specificity of 100 % for the flocktype AIV Ab.

4.2.2 Specificity for AIV-negative field samples

Procedure

To further evaluate specificity, altogether *n* = 371 pre-selected field sera from chickens with a negative result in the BioChek AIV ELISA (BioChek BV, Reeuwijk, The Netherlands) were tested with the flocktype AIV Ab.

Results

Distribution of *n* = 371 AIV-antibody negative field sera is shown in Figure 2. Mean value of S/P ratios is 0.017 ± 0.024. The mean value of the S/P-ratios is 17.85-times from the cut-off (S/P = 0.3).

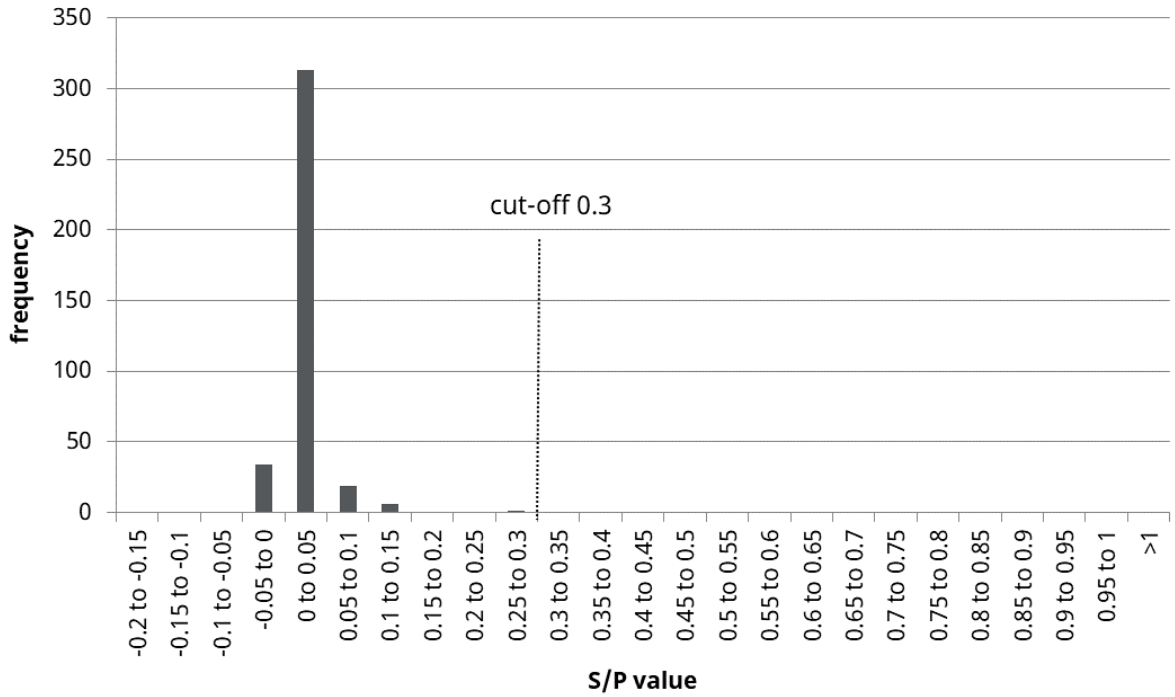

Figure 2. Frequency distribution of the S/P values of *n* = 371 AIV-antibody negative field samples tested with the flocktype AIV Ab

4.3 H5N2 infection study

Procedure

Altogether *n* = 21 chickens were infected with AIV A/chicken/Italy/8/98 (H5N2) and bled 21 days post infection. Samples were kindly provided by the Laboratory for Avian Influenza, at the FLI Riems, Germany.

Results

All *n* = 21 infection sera scored positive with the flocktype AIV Ab (Figure 3). The mean value of S/P-ratios is 1.322 ± 0.591.

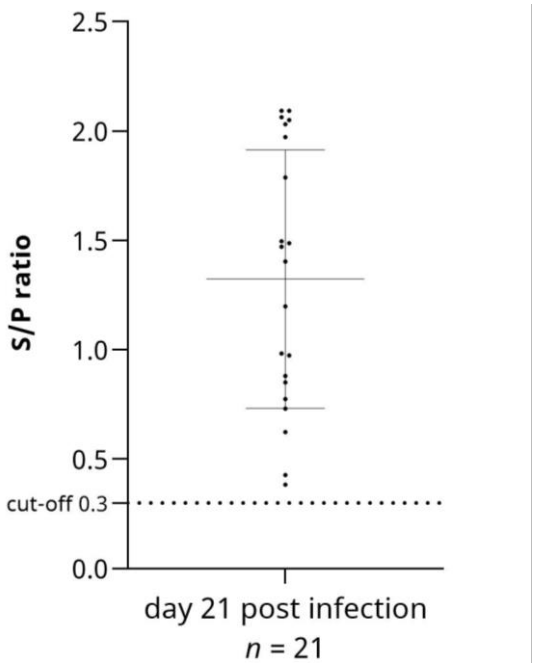

Figure 3. Results of the H5N2 infection study samples tested with the flocktype AIV Ab (bar: Mean with standard deviation)

Conclusion

The flocktype AIV Ab constantly detects AIV-specific antibodies from infection.

4.4 H7N1 vaccination and challenge study

Procedure

Chickens were vaccinated with the recombinant AIV vaccine ILTV-H7 and subsequently challenged. The vaccine ILTV-H7 is an *Infectious Laryngotracheitis Virus* with an insertion of the AIV hemagglutinin H7 gene from AIV A7/chicken/Italy/445/99 (H7N1). The animals were challenged with AIV A7/chicken/Italy/445/99 (H7N1). The control group „d 0“ (*n* = 8) was bled 15 days post vaccination without challenge. After challenging, samples were taken at days 3 (*n* = 2), 6 (*n* = 2), 19 (*n* = 10) and were tested in the flocktype AIV Ab. Samples were kindly provided by the Laboratory for Avian Influenza, at the FLI Riems, Germany.

Results/ Conclusion

Study results are depicted in Figure 4. The control group „d 0“ was bled 15 days post vaccination with the recombinant vaccine without challenge. Sera from these animals give true negative results in the flocktype AIV Ab since the recombinant vaccine ILTV-H7 only induces AIV-specific antibodies to AIV hemagglutinin H7, which is not the coating antigen of the flocktype AIV Ab. Antibodies against AIV H7N1 are detected from day 6 post challenge with the challenge virus AIV A7/chicken/Italy/445/99. Taken together, the flocktype AIV Ab is a reliable tool for early detection of AIV-specific antibodies after challenge infection.

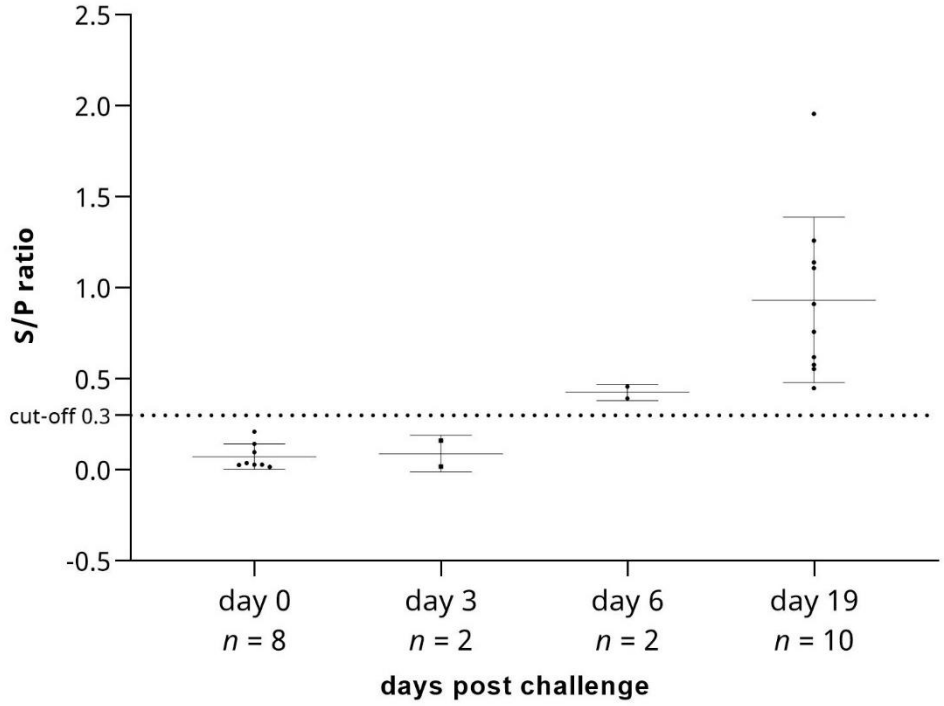

Figure 4. Results of the H7N1 vaccination and challenge study samples tested with the flocktype AIV Ab (bar: Mean with standard deviation)

4.5 Receiver Operating Characteristics (ROC)

Definition

Receiver Operating Characteristics (ROC) curve analysis is used to compare the diagnostic performance of two or more diagnostic tests. In the ROC curve the true positive rate (sensitivity) is plotted versus the false positive rate (1-specificity) for all possible cut-off values of the flocktype AIV Ab ELISA. Each point on the ROC plot represents a sensitivity/ specificity pair corresponding to a particular cut-off value. The resulting area under the curve (AUC) of the ROC-function quantifies the correlation of the test results. An AUC of 1 reflects a perfect differentiation of “true positive” and “true negative” in both tests. The closer the AUC converges to 1, the higher is the overall test correlation.

Procedure

During validation, 536 serum and plasma samples (from vaccination and infection experiments, breeding animals as well as field samples; Table 2) of known AIV-infection status were tested using the flocktype AIV Ab. Status of the samples was assessed by HI test, AGP, BioChek, or IDEXX AIV ELISAs.

A ROC analysis was performed to determine the optimal cut-off value for the flocktype AIV Ab ELISA according to the predefined status of the samples. ROC-Plot and the AUC value were used to demonstrate the agreement (Figure 5). Results were calculated using the software MATLAB®.

Table 2. Description of the tested serum samples for ROC analysis

Sample group	**Quantity**
AIV-antibody positive serum samples	104
AIV-antibody negative serum samples	**432**
total	**536**

Results

By ROC analysis (Figure 5), an AUC of 0.9772 was determined which implies an excellent correlation of the infection status for the analyzed samples.

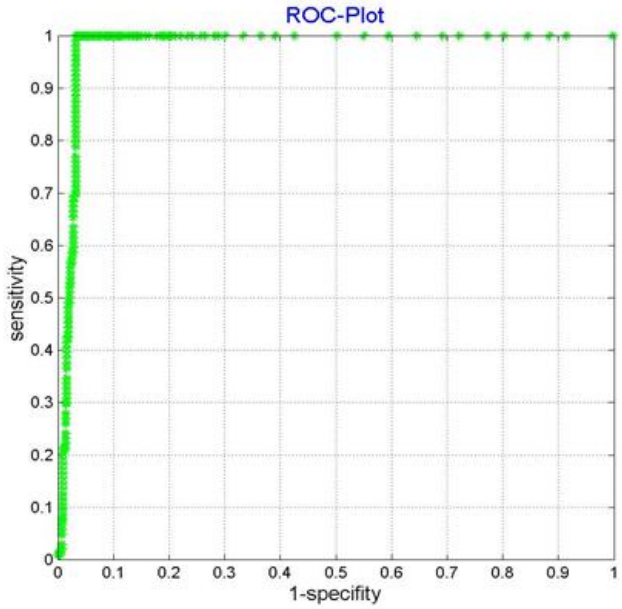

Figure 5. ROC plot for flocktype AIV Ab

Kappa is a measure of reliability. It indicates the proportion of agreement between two tests [Cohen *et* al.]. If κ > 0.75 the test is excellent [Landis *et* al.]). Accordingly, the S/P value at κ_{max} reflects the optimal sensitivity and specificity by taking the lowest false positive/negative rate into account. At κ_{max} = 0.9205 and the respective S/P value between 0.269 – 0.313, sensitivity of 100 % and specificity of 96.8 % were calculated (Figure 6; data not shown).

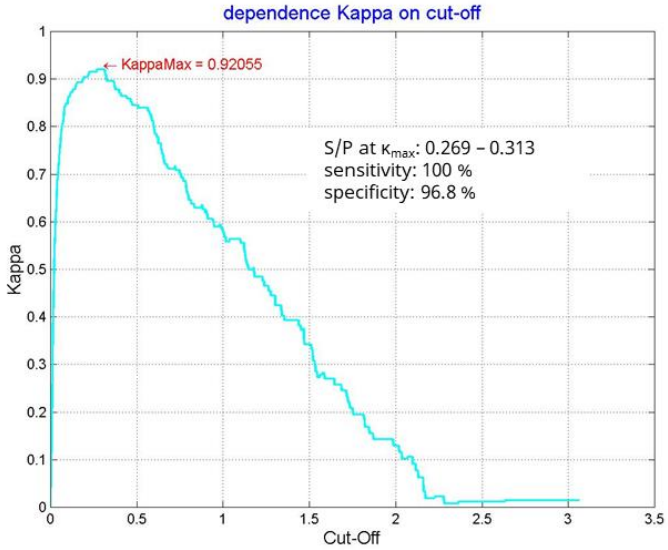

Figure 6. Dependence of κ on cut-off for flocktype AIV Ab

Conclusion

Results of the flocktype AIV Ab were in excellent correlation to the status of the samples. The following cut-off was defined:

- Samples with S/P values < 0.3 are negative.
- Samples with S/P values ≥ 0.3 are positive.

4.6 Real time stability

Procedure

To demonstrate the storage stability of the flocktype AIV Ab, test kits (batch F201800018) were stored according to the manufacturer's instructions at 4°C and tested after 24 months using a quality control (QC) sample set consisting of *n* = 16 sera as well as one titration series. The S/P values obtained were compared to the results of the batch control which was performed directly after the kit production.

Results

Obtained S/P values for the *n* = 16 QC samples as well as the titration series in the batch control, and after 24 months of storage are shown in Table 3 and Figure 7. The mean S/P values of the antibody-positive samples increased by 3.9 % after 24 months of storage and the influence on the S/P-values of the positive samples is negligible (+1.2 %). In no case the sample classification changed.

Conclusion

The flocktype AIV Ab kit demonstrated an excellent stability.

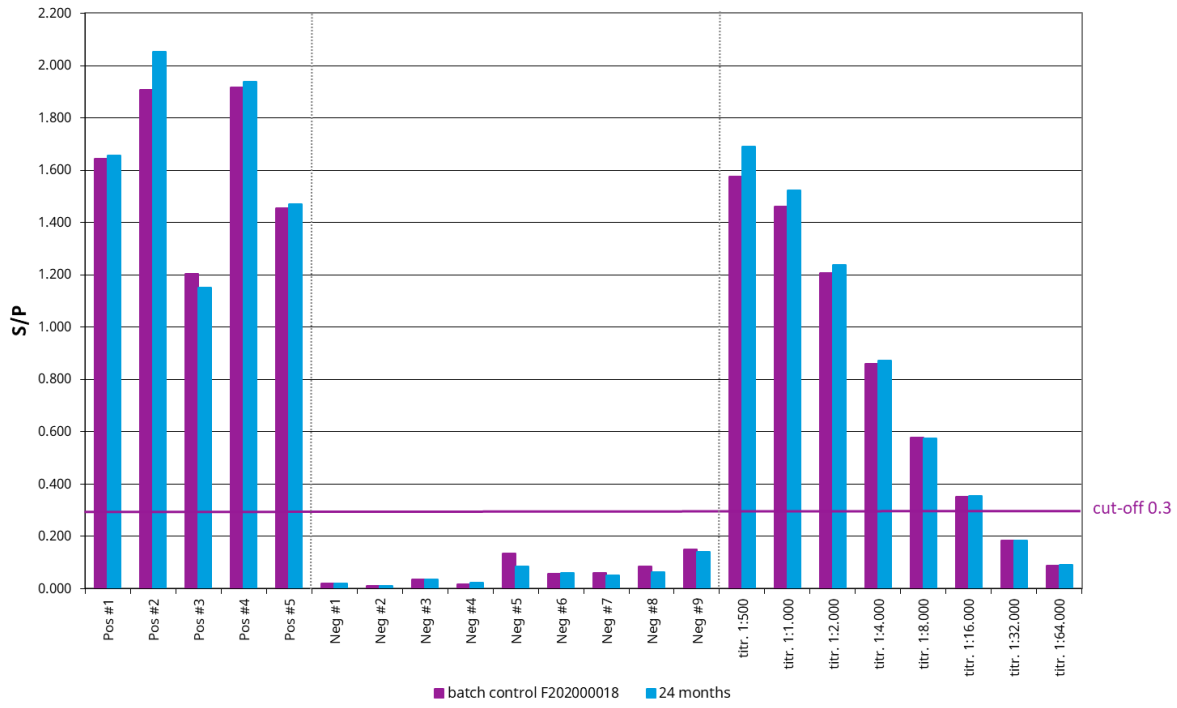

Figure 7. Real time stability data (S/P values, QC sample set) for the flocktype AIV Ab

Table 3. Results of the flocktype AIV Ab (batch F201800018) in a real time stability test (24 months)

Sample		Batch control					24 months storage (4°C)					Variation [%]	
		OD	S/P	Titer	TG	Res.	OD	S/P	Titer	TG	Res.	OD	S/P
Neg Control [MV]		0.062					0.049					-21.0	
Pos Control [MV]		1.522					1.538					1.1	
Positive samples	Pos 1	2.426	1.643	12650	10	**pos**	2.517	1.657	12822	10	**pos**	3.7	0.9
	Pos 2	2.812	1.909	15934	11	**pos**	3.106	2.053	17828	12	**pos**	10.4	7.6
	Pos 3	1.800	1.204	7841	7	**pos**	1.763	1.151	7313	7	**pos**	-2.0	-4.4
	Pos 4	2.823	1.916	16028	12	**pos**	2.937	1.940	16333	12	**pos**	4.0	1.2
	Pos 5	2.163	1.456	10504	9	**pos**	2.236	1.469	10644	9	**pos**	3.4	0.9
MV positive samples												**3.9**	**1.2**
Negative samples	Neg 1	0.089	0.021	16	0	neg	0.078	0.019	14	0	neg	-12.0	-8.3
	Neg 2	0.075	0.011	6	0	neg	0.065	0.011	5	0	neg	-13.3	-5.7
	Neg 3	0.110	0.036	35	0	neg	0.104	0.037	37	0	neg	-5.7	3.0
	Neg 4	0.080	0.018	12	0	neg	0.084	0.024	18	0	neg	5.0	33.8
	Neg 5	0.251	0.134	266	0	neg	0.178	0.087	136	0	neg	-29.0	-35.3
	Neg 6	0.148	0.059	75	0	neg	0.140	0.061	80	0	neg	-5.1	3.9
	Neg 7	0.152	0.062	81	0	neg	0.125	0.051	60	0	neg	-17.8	-17.5
	Neg 8	0.186	0.084	129	0	neg	0.143	0.063	84	0	neg	-22.9	-24.6
	Neg 9	0.287	0.150	316	0	neg	0.260	0.142	290	0	neg	-9.5	-5.3
MV negative samples												-12.3	-6.2
Titration series	1:500	2.332	1.575	11854	9	**pos**	2.567	1.691	13224	10	**pos**	10.1	7.4
	1:1.000	2.165	1.461	10553	9	**pos**	2.318	1.524	11265	9	**pos**	7.1	4.3
	1:2.000	1.804	1.208	7880	7	**pos**	1.890	1.236	8164	8	**pos**	4.7%	2.3
	1:4.000	1.306	0.861	4678	5	**pos**	1.350	0.874	4783	5	**pos**	3.4	1.5
	1:8.000	0.897	0.578	2530	3	**pos**	0.905	0.575	2510	3	**pos**	0.9	-0.5
	1:16.000	0.571	0.352	1182	1	**pos**	0.575	0.353	1186	1	**pos**	0.7	0.2
	1:32.000	0.327	0.185	437	0	neg	0.322	0.183	432	0	neg	-1.5	-0.7

1:64.000	0.187	0.088	140	0	neg	0.183	0.090	144	0	neg	-2.1	1.8

Results: pos = positive, neg = negative
MV = mean value, TG = Titer group, Res = Result

4.7 Reproducibility

4.7.1 Homogeneity of the coated test plates (intra-plate variance)

Procedure

In order to determine the test plate homogeneity for the flocktype AIV Ab a positive serum sample was analyzed over the whole test plate (batch F202000131). The mean values of the OD (MV), the standard deviation (SD) and the coefficient of variation (CV) were calculated.

Results

The well-to-well variation (CV) was 3.5 % (Table 4).

Table 4. Test results regarding homogeneity of the flocktype AIV Ab

	1	2	3	4	5	6	7	8	9	10	11	12	MV	SD	CV
A	1.111	1.104	1.131	1.113	1.094	1.087	1.076	1.073	1.077	1.073	1.099	1.061	1.09	0.02	1.9
B	1.117	1.083	1.071	1.084	1.089	1.077	1.064	1.085	1.047	1.077	1.052	1.055	1.08	0.02	1.8
C	1.087	1.077	1.062	1.082	1.075	1.075	1.056	1.042	1.045	1.079	0.990	1.064	1.06	0.03	2.5
D	1.124	1.048	1.079	1.069	1.080	1.051	1.047	1.059	1.024	1.041	1.011	1.055	1.06	0.03	2.8
E	1.193	1.133	1.096	1.106	1.123	1.099	1.092	1.115	1.071	1.064	1.035	1.066	1.10	0.04	3.7
F	1.122	1.049	1.051	1.057	1.111	1.066	1.036	1.036	1.027	1.019	0.989	1.030	1.05	0.04	3.5
G	1.082	1.059	1.062	1.033	1.088	1.087	1.026	1.020	1.005	0.999	0.983	1.001	1.04	0.04	3.6
H	1.138	1.086	1.084	1.089	1.123	1.051	1.067	1.077	1.052	1.046	0.986	1.016	1.07	0.04	3.9
MV	1.122	1.080	1.080	1.079	1.098	1.074	1.058	1.063	1.044	1.050	1.018	1.044			
SD	0.034	0.029	0.025	0.026	0.019	0.017	0.021	0.031	0.024	0.029	0.041	0.025			
CV	3.1	2.7	2.3	2.4	1.7	1.6	2.0	2.9	2.3	2.8	4.1	2.4			

MV = mean value; SD = standard deviation; CV = coefficient of variation (given in %)

Total: MV = 1.067; SD = 0.037; CV = 3.5 %

Conclusion

The homogeneity of the flocktype AIV Ab test plates is very good.

4.7.2 Repeatability (Inter-plate variance)

Procedure

Together, six AIV-antibody positive samples and six AIV-antibody negative samples were analyzed on three different plates of the same batch (F201800018). The mean S/P values (MV), the standard deviation (SD) and the coefficient of variation (CV) were calculated (Table 5).

Results

As shown in Table 5 the average inter-plate variation (CV) of the flocktype AIV Ab for AIV-antibody positive samples is 3.8 %.

Table 5. Repeatability (inter-plate variation) of the flocktype AIV Ab in three different plates (batch F201800018)

Samples		**S/P values obtained with plates**			**S/P**		
		Plate 1	**Plate 2**	**Plate 3**	**MV**	**SD**	**CV [%]**
Positive samples	Pos sample #1	1.868	1.874	1.984	1.909	0.065	3.4
	Pos sample #2	1.592	1.576	1.761	1.643	0.103	6.2
	Pos sample #3	1.471	1.422	1.475	1.456	0.030	2.0
	Pos sample #4	1.238	1.156	1.219	1.204	0.043	3.6
	Pos sample #5	1.872	1.898	1.978	1.916	0.055	2.9
	Pos sample #6	1.894	1.912	2.055	1.954	0.089	4.5
MV positive samples					1.680	0.064	**3.8**
Negative samples	Neg sample #1	0.002	0.023	0.009	0.011	0.010	91.8
	Neg sample #2	0.028	0.044	0.036	0.036	0.008	22.7
	Neg sample #3	0.032	0.056	0.042	0.043	0.012	27.9
	Neg sample #4	0.106	0.148	0.147	0.134	0.024	17.9
	Neg sample #5	0.036	0.059	0.048	0.047	0.012	24.4
	Neg sample #6	0.009	0.026	0.030	0.021	0.011	52.5
MV negative samples					0.049	0.013	39.5

MV = mean value; CV = coefficient of variation

Conclusion

The reproducibility of the results obtained with the flocktype AIV Ab is excellent.

4.7.3 Batch-to-batch variation

Procedure

Altogether *n* = 20 AIV-antibody positive serum samples and *n* = 18 AIV-antibody negative serum samples were tested with three different batches (254111033, 251111017, 248110508) of the flocktype AIV Ab. The mean OD and S/P values on different batches, the standard deviation (SD) and the coefficients of variation (CV) were calculated (Table 6).

Results/ Conclusion

For the flocktype AIV Ab an average batch-to-batch variation (CV) of 12.0 % for AIV-antibody positive samples was determined (Table 6). In no case the sample classification changed. A high degree of reproducibility of results using different flocktype AIV Ab batches is therefore guaranteed.

Table 6. Batch-to-batch variation of the flocktype AIV Ab for three different batches

Samples		S/P values obtained with batches			S/P		
		254111033	**251111017**	**248110508**	**MV**	**SD**	**CV [%]**
Positive samples	Pos sample #1	0.853	0.852	0.50	0.735	0.204	27.7
	Pos sample #2	0.765	0.850	0.79	0.800	0.044	5.5
	Pos sample #3	0.433	0.433	0.42	0.427	0.010	2.3
	Pos sample #4	1.363	1.399	1.33	1.363	0.035	2.6
	Pos sample #5	1.697	2.221	1.96	1.960	0.262	13.4
	Pos sample #6	1.546	1.864	1.67	1.694	0.160	9.4
	Pos sample #7	1.357	1.674	1.55	1.526	0.160	10.5
	Pos sample #8	1.138	1.345	1.17	1.216	0.112	9.2
	Pos sample #9	1.880	2.398	2.18	2.151	0.260	12.1
	Pos sample #10	1.338	1.699	1.49	1.509	0.181	12.0
	Pos sample #11	1.905	2.430	2.19	2.174	0.263	12.1
	Pos sample #12	1.395	1.650	1.82	1.621	0.213	13.1
	Pos sample #13	1.724	2.465	2.20	2.131	0.375	17.6
	Pos sample #14	1.458	1.805	1.67	1.643	0.175	10.6
	Pos sample #15	0.545	0.586	0.43	0.520	0.081	15.6
	Pos sample #16	1.328	1.642	1.26	1.409	0.204	14.5
	Pos sample #17	1.511	2.002	1.64	1.716	0.256	14.9
	Pos sample #18	0.907	1.116	0.85	0.956	0.142	14.8
	Pos sample #19	1.555	2.015	1.65	1.739	0.243	14.0
	Pos sample #20	1.581	1.888	1.75	1.740	0.154	8.8
MV positive samples					1.452	0.177	**12.0**

Negative samples	Neg sample #1	0.046	0.061	0.06	0.057	0.009	16.3
	Neg sample #2	0.022	0.022	0.00	0.015	0.012	83.5
	Neg sample #3	0.030	0.026	0.02	0.024	0.007	28.1
	Neg sample #4	0.020	0.015	0.00	0.012	0.010	83.7
	Neg sample #5	0.021	0.012	0.00	0.012	0.009	74.3
	Neg sample #6	0.027	0.015	0.01	0.018	0.008	45.2
	Neg sample #7	0.011	0.030	0.02	0.019	0.010	51.1
	Neg sample #8	0.012	0.007	0.00	0.007	0.005	76.3
	Neg sample #9	-0.008	-0.011	-0.02	-0.014	0.008	-56.8
	Neg sample #10	0.021	0.016	-0.01	0.010	0.015	144.8
	Neg sample #11	0.117	0.117	0.09	0.108	0.016	14.4
	Neg sample #12	-0.001	0.005	0.00	0.001	0.004	600.9
	Neg sample #13	0.044	0.036	0.04	0.041	0.004	10.7
	Neg sample #14	0.070	0.083	0.10	0.085	0.015	18.1
	Neg sample #15	0.064	0.052	0.05	0.056	0.007	11.7
	Neg sample #16	0.018	0.012	0.00	0.010	0.010	108.2
	Neg sample #17	0.027	0.025	0.02	0.025	0.002	7.1
	Neg sample #18	0.054	0.048	0.04	0.046	0.008	18.3
MV negative samples					0.030	0.009	74.2

MV = mean value; SD = standard deviation, CV = coefficient of variation

4.8 Deventer International Proficiency Testing Scheme for Avian Influenza (AIV) antibody detection in serum 2017

Procedure

The samples from the 2017 Deventer International Proficiency Testing Scheme (PTS) for Avian Influenza (AIV) antibody detection in serum were analyzed in two independent test runs with the flocktype AIV Ab.

Results

The results are shown in Table 7. All samples scored correctly with the flocktype AIV Ab with comparable quantitative results in both test runs.

Table 7. Results of the Deventer PTS 2017 for Avian Influenza analyzed with the flocktype AIV Ab in two test runs

Samples			Test run 1					Test run 2				
No.	Information	Status	MV OD	MV S/P	Titer	TG	Result	MV OD	MV S/P	Titer	TG	Result
#1	pooled serum from 8-week-old SPF broilers inoculated with LP strain H5N2: A/Chicken/Belgium150/99 - 28 dpi	pos	1.330	0.867	4727	5	pos	1.217	0.827	4397	5	pos
#2	mixed serum sample from 46-week-old SPF layers and 17-week-old SPF broiler breeders infected with LP H5N2 and H9N2 respectively - 21 dpi	pos	1.903	1.254	8345	8	pos	1.936	1.346	9309	8	pos
#3	pooled serum from 5-week-old SPF layers that had been inoculated with a LP H9N2 strain – 14 dpi	pos	2.057	1.358	9431	8	pos	2.072	1.444	10370	9	pos
#4	pooled serum from 7-week-old SPF layers	neg	0.021	-0.016	0	0	neg	0.023	-0.034	0	0	neg
#5	pooled serum sample from 35-day-old SPF turkeys inoculated with LP H7N1 - 9 dpi	pos	1.993	1.315	8973	8	pos	2.031	1.415	10048	9	pos
#6	pooled serum from 31-day-old SPF broiler breeders that had been inoculated with a LP H7N7 strain - 22 dpi	pos	2.532	1.679	13076	10	pos	2.472	1.733	13733	10	pos
#7	pooled serum from 12-week-old SPF broiler breeders that have been inoculated with LP H5N3 - 21 dpi	pos	0.995	0.641	2970	3	pos	1.014	0.681	3257	4	pos
#8	pooled sample from 8-week-old SPF Layers infected with LP strain H6N1 A/Chicken/Netherlands/SP917/2010 - 21 dpi	pos	2.582	1.712	13483	10	pos	2.576	1.808	14659	11	pos

MV = mean value; dpi = days post infection; dpv = days post vaccination

5 References

- Cohen J. (1960) A coefficient of agreement for nominal scales. Educ. Psych. Meas., 20, 37-46
- Landis J.R., Koch G.G. (1977) The measurement of observer agreement for categorical data. Biometrics, 33, 159-174

December 2022 | EN

flocktype® AIV Ab
Handbook

For detection of antibodies to Avian Influenza A Virus

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 435

REF 2 plates (cat. no. FT274012)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

flocktype AIV Ab **Cat. no.** **Number of plates**	**(2)** **FT274012** **2**
Test Plate: microtiter plate with 96 wells, coated with non-infectious AIV antigen	2
Sample Diluent, ready to use	1 x 125 ml
Negative Control, ready to use	1 x 3.5 ml
Positive Control, ready to use	1 x 3.5 ml
Wash Buffer, 10x concentrate	1 x 125 ml
Conjugate, ready to use	1 x 24 ml
TMB Substrate, ready to use	1 x 24 ml
Stop Solution, ready to use	1 x 24 ml
Handbook	1

Intended use

The flocktype AIV Ab is a specific and sensitive ELISA for detecting antibodies to Avian Influenza A Virus in serum and plasma samples from chicken and turkey.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 435) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For chicken and turkey samples

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of flocktype AIV Ab is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the flocktype AIV Ab ELISA should be stored at 2-8°C and are stable until the expiration date stated on the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18-25°C) to avoid salt crystallization. If test strips are provided with the kit, store the remaining test strips in the re-sealed foil pouch with desiccant at 2-8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The flocktype AIV Ab is a highly sensitive and specific solution for the detection of antibodies to Avian Influenza A Virus (AIV) in avian serum and plasma samples.

Avian influenza is caused by various strains of Influenza A Virus. It infects wild fowl as well as domestic poultry. Influenza A Virus strains are classified as low pathogenic or highly pathogenic. Highly pathogenic strains belong to subtypes H5 or H7 and can cause the severe systemic symptoms known as bird flu or avian flu. The flocktype AIV Ab uses a structural protein of AI virus prepared by recombinant technology as antigen. This protein is highly conserved amongst AIV strains and strongly immunogenic. Thus, all subtypes of Influenza A viruses will be detected.

Principle

The microtiter test plate is coated with a recombinant structural protein from the virus. During sample incubation AIV-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing.

The anti-IgY-HRP conjugate detects serum antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 15 minutes. In the presence of AIV-specific antibodies, within the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution.

The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of anti-AIV antibodies in the sample.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.

Things to do before starting

- Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.
- Dilute Wash Buffer (10x) 1:10 in distilled water. For example, for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.
- Serum/ plasma samples: Prior to sample analysis, with serum/plasma samples, dilute 1:500 in Sample Diluent (e.g., dilute 1 µl sample in 499 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtitre plates for dilution. Change pipet tips for each sample.

  Alternatively, serum/plasma samples can be diluted from a pre-dilution (1:50 in Sample Diluent) directly in the Test Plate (see Procedure step 1a).
- Controls are ready to use and do not require a dilution.

Protocol: ELISA

Please read „Things to do before starting“, page 9.

Procedure

1. Pipet 100 µl of each of the ready-to-use Negative Control (in duplicates) and Positive Control (in duplicates) and the 1:500 samples into the Test Plate wells.

1a. Alternatively, pipet 90 µl of Sample Diluent in each sample well and add 10 µl of the of the 1:50 pre-diluted sample. Mix well.

   Note: Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

2. Incubate for 30 min at room temperature (18-25°C).
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
5. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).
6. Remove solution from wells by aspiration or tapping.
7. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 15 min at room temperature in the dark. Begin timing after the first well is filled.
10. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.

11. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.7.
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.2.

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the MV of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Endpoint titers are calculated from the S/P ratio at a 1:500 dilution using the following equation:

$$Log_{10}\ Titer = 1.54\ (Log_{10}\ S/P) + 3.77$$

Interpretation of the results

- Samples with the S/P ratio < 0.3 are negative. Specific antibodies to AIV could not be detected.
- Samples with the S/P ratio ≥ 0.3 are positive. Specific antibodies to AIV were detected.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.
Visit **www.indical.com** for more information about afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Limited License Agreement for flocktype AIV Ab

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Change index

Handbook	Version	Change
HB-1589-EN-004	December 2022	Editorial changes
HB-1589-EN-003	June 2022	Change TMB incubation time to 15 min
HB-1589-002	May 2018	INDICAL design

Quick guide for flocktype AIV Ab

Sample dilution:

Serum, plasma 1:500, mix well

Step	Protocol
1. Sample	100 µl/ well
2. Incubation	30 min at RT
3. Wash	3 x 300 µl
4. Conjugat	100 µl/ well
5. Incubation	30 min at RT
6. Wash	3 x 300 µl
7. TMB	100 µl/ well
8. Incubation	15 min at RT
9. Stop	100 µl/ well
10. Read	450 nm

Data interpretation

	Negative	Positive
Serum, plasma	S/P < 0.3	S/P ≥ 0.3

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Contents

Sample extraction solutions

Our sample extraction solutions are specifically designed to facilitate accurate and efficient testing in your laboratory.

Sample collection

Ordering information

Product	Format	Cat. No.
GenoTube Livestock Swab	1 swab	9062010

Sample extraction kits for molecular biology workflow

Ordering information

Product	Format	Cat. No.
Extraction kits		
MagMAX CORE Nucleic Acid Purification Kit	100 reactions	A32700
	500 reactions	A32702
MagMAX CORE Mechanical Lysis Module	100 reactions	A32836
MagMAX CORE Nucleic Acid Purification Kit + Mechanical Lysis Module	100 reactions	A37487
MagMAX CORE Nucleic Acid Purification Kit + Mechanical Lysis Module + Glass Microbeads	100 reactions	A37488
MagMAX CORE Nucleic Acid Purification Kit with Mastitis and Panbacteria Module	100 reactions	A40289
MagMAX Pathogen RNA/DNA Kit	480 reactions	4462359
MagMAX Total Nucleic Acid Isolation Kit	100 reactions	AM1840
MagMAX Viral RNA Isolation Kit	50 reactions	AM1939
MagMAX-96 AI/ND Viral RNA Isolation Kit	384 reactions	AM1835
MagMAX-96 Viral RNA Isolation Kit	5 x 96 reactions	AMB18365
	96 reactions	AM1836
MagMAX Viral RNA/DNA Extraction Prefilled Kit (China)	2 x 96 reactions	A52990
Sample extraction reagents and consumables		
Carrier RNA	500 µL	4382878
Magnetic Stand-96, Standard Plate	1 each	AM10027
Magnetic Stand-96, U-Bottom Plate	1 each	AM10050
Magnetic Stand, Single Tube	1 each	AM10026
PK Buffer for MagMAX-96 DNA Multi-Sample Kit	96 reactions	4489111
MagMAX CORE Glass Microbeads	100 reactions	A37489
MagMAX CORE Lysis Solution	50 mL	A32837

For Research Use Only. Not for use in diagnostic procedures.

Note: All products listed as PCR are qPCR products.

Sample extraction consumables

Ordering information

Product	Format	Cat. No.
Sample extraction		
6-Tip Combs for KingFisher Duo Prime system	48 combs	97003510
12-Tip Combs for KingFisher Duo Prime system	50 combs	97003500
Combi Pack for KingFisher Duo Prime system (tip combs, plates, and elution strips)	96-sample pack	97003530
Elution Strips for KingFisher Duo Prime system	40 strips	97003520
KingFisher 96 KF Microplates (200 µL)	48 plates	97002540
KingFisher 96 Tip Comb for KF Magnets	100 combs	97002534
KingFisher Flex 24 Deep-Well Magnetic Head	1 head	24074440
KingFisher Flex 24 Deep-Well Plates	50 plates	95040480
KingFisher Flex 24 Deep-Well Tip Combs and Plates	50 combs/plates	97002610
KingFisher Flex 96 Deep-Well Magnetic Head	1 head	24074430
KingFisher Flex Microtiter Deep-Well 96 Plates	50 plates	95040460
KingFisher mL Combi Pack (tip combs and tubes)	240-sample pack	97002141
KingFisher mL Tip Combs	800 combs	97002111
KingFisher mL Tubes	900 tubes	97002121

Master mixes

Ordering information

Product	Format	Cat. No.
VetMAX Fast Multiplex Master Mix, with ROX	100 reactions	A57081
	500 reactions	A57305
	1,000 reactions	A57306
AgPath-ID One-Step RT-PCR Reagents	100 reactions	AM1005
	500 reactions	4387424
	1,000 reactions	4387391
Detection Enhancer	100 reactions	A44810
	500 reactions	A44941
	1,000 reactions	A44811
Path-ID Multiplex One-Step RT-PCR Kit	100 reactions	4442135
	500 reactions	4442136
	1,000 reactions	4442137
Path-ID qPCR Master Mix	100 reactions	4388643
	500 reactions	4388644
VetMAX-Plus Multiplex One-Step RT-PCR Kit	100 reactions	4415330
VetMAX-Plus RT-PCR Kit	100 reactions	4415328
VetMAX-Plus qPCR Master Mix	100 reactions	4415327

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Note: All products listed as PCR are qPCR products.

Internal positive controls (IPCs)

Ordering information

Product	Format	Cat. No.
VetMAX Xeno Internal Positive Control RNA	100 reactions	A29763
	500 reactions	A29761
VetMAX Xeno Internal Positive Control DNA	100 reactions	A29764
	500 reactions	A29762
VetMAX Xeno Internal Positive Control, LIZ Assay	100 reactions	A29766
	500 reactions	A29768
VetMAX Xeno Internal Positive Control, VIC Assay	100 reactions	A29765
	500 reactions	A29767

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Note: All products listed as PCR are qPCR products.

Diagnostic testing solutions

Our comprehensive range of diagnostic testing solutions are designed to address diverse needs and help ensure accurate and efficient diagnoses in animals.

Ruminant—bovine/ovine/caprine

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Abortive/reproductive pathogens				
Anaplasma phagocytophilum, bovine herpesvirus type 4, *Campylobacter fetus*, *Chlamydophila* spp., *Coxiella burnetii*, *Listeria monocytogenes*, pathogenic *Leptospira* spp., *Salmonella* spp.	VetMAX Ruminant Abortion Screening Kit Multiplex Detection	**PCR**	25 reactions	SARP
Bluetongue virus (BTV)	VetMAX European BTV Typing Kit	**PCR**	8 x 50 tests	BTVEUG
Bluetongue virus 1 (BTV1)	VetMAX BTV1 IAH Typing Kit	**PCR**	50 reactions	BTV1GIAH50
Bluetongue virus 4 (BTV4)	VetMAX BTV4 IAH Typing Kit	**PCR**	50 reactions	BTV4GIAH50
Bluetongue virus 8 (BTV8)	VetMAX BTV8 IAH Typing Kit	**PCR**	50 reactions	BTV8GIAH50
Bovine herpesvirus type 1 (BHV-1)	PrioCHECK BHV-1-GB Kit	**ELISA**	5 x 96-well plates	7610359
Bovine viral diarrhea virus (BVDV)	PrioCHECK Ruminant BVD & BD Ab Milk Kit	**ELISA**	5 x 96-well plates	BVDAL5
	PrioCHECK Ruminant BVD p80 Ab Serum & Milk Kit	**ELISA**	5 x 96-well plates	BVDIL105
	PrioCHECK Bovine BVDV Ab Plate Kit	**ELISA**	5 x 96-well plates	7588940
	PrioCHECK Bovine BVDV PIfocus Ag Strip Kit	**ELISA**	900 samples	7610140
	VetMAX BVDV 4ALL Kit	**PCR**	100 reactions	BVD4ALL
Brucella abortus and *Brucella melitensis*	PrioCHECK Brucella Antibody 2.0 ELISA Kit, strip	**ELISA**	5 x 96-well plates	7610700
	PrioCHECK Brucella Antibody 2.0 ELISA Kit, solid	**ELISA**	5 x 96-well plates	7610710
Campylobacter fetus	VetMAX *C. fetus* Kit	**PCR**	50 reactions	CFP50
Chlamydophila spp.	VetMAX *Chlamydophila* spp. Kit	**PCR**	50 reactions	CHLP50
Coxiella burnetii	PrioCHECK Ruminant Q Fever Ab Plate Kit	**ELISA**	2 x 96-well plates	ELISACOXLS2
		ELISA	5 x 96-well plates	ELISACOXLS5
	VetMAX *C. burnetii* Absolute Quant Kit	**PCR**	100 reactions	FQPAQ
Coxiella burnetii, *Chlamydophila* spp.	VetMAX *C. burnetii* & *Chlamydophila* spp. Kit	**PCR**	100 reactions	TFQQCHP
Leptospira hardjo	PrioCHECK *L. hardjo* Ab Strip Kit	**PCR**	5 x 96-well plates	7442080
	PrioCHECK *L. hardjo* Ab Plate Kit	**ELISA**	50 x 96-well plates	7442090
Neospora caninum	VetMAX *Neospora caninum* Kit	**PCR**	50 reactions	NEOSP
Salmonella dublin	PrioCHECK *S. dublin* Ab Strip Kit	**ELISA**	5 x 96-well plates	7610640
Salmonella dublin and *S. typhimurium*	PrioCHECK Bovine Salmonella Ab Strip Kit	**ELISA**	5 x 96-well plates	7610620
Schmallenberg virus	VetMAX Schmallenberg Virus Kit	**PCR**	50 reactions	SBVS50
Toxoplasma gondii	VetMAX *T. gondii* Kit	**PCR**	50 reactions	TXP50

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Note: All products listed as PCR are qPCR products.

Ruminant—bovine/ovine/caprine (continued)

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Bovine mastis				
Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, Streptococcus uberis	VetMAX MastiType Micro4 Kit	**PCR**	100 reactions	A39235
	VetMAX MastiType Micro4 Combo Kit (MagMAX CORE Nucleic Acid Purification Kit with Mastitis and Panbacteria Module, A40289 + A39235)	**PCR**	100 reactions	A40292
Staphylococcus aureus, Staphylococcus spp. (including all major coagulase-negative staphylococci), *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli, Enterococcus* spp. (including *E. faecalis* and *E. faecium), Klebsiella oxytoca* (and/or *K. pneumoniae), Serratia marcescens, Corynebacterium bovis, Trueperella pyogenes* and/or *Peptoniphilus indolicus, Staphylococcal β-lactamase* gene (penicillin-resistance gene), *Mycoplasma bovis, Mycoplasma* spp., yeast, *Prototheca* spp.	VetMAX MastiType Multi Kit	**PCR**	100 reactions	A39227
	VetMAX MastiType Multi Combo Kit (MagMAX CORE Nucleic Acid Purification Kit with Mastitis and Panbacteria Module, A40289 + A39227)	**PCR**	100 reactions	A40290
Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, Mycoplasma spp., *Mycoplasma alkalescens, Mycoplasma bovigenitalium, Mycoplasma californicum, Mycoplasma canadense*	VetMAX MastiType Myco8 Kit	**PCR**	100 reactions	A39236
	VetMAX MastiType Myco8 Combo Kit (MagMAX CORE Nucleic Acid Purification Kit with Mastitis and Panbacteria Module, A40289 + A39236)	**PCR**	100 reactions	A40291
Bovine tuberculosis				
Mycobacterium bovis	BOVIGAM 2G TB Kit	**ELISA**	10 x 96-well plates	63330
	BOVIGAM TB Kit	**ELISA**	10 x 96-well plates	63320
		ELISA	30 x 96-well plates	63326
	BOVIGAM PC-EC Stimulating Antigen (lyophilized)	**ELISA**	120 samples	7600100
	BOVIGAM PC-HP Stimulating Antigen (lyophilized)	**ELISA**	120 samples	7600105
	BOVIGAM Pokeweed Mitogen	**ELISA**	3.2 mg	5108777
	BOVIGAM Tuberculin PPD Stimulating Antigen, bovine	**ELISA**	5 mL; 30,000 IU/mL	7600060
	BOVIGAM Tuberculin PPD Stimulating Antigen, avian	**ELISA**	5 mL; 25,000 IU/mL	7600065
Mycobacterium tuberculosis complex	VetMAX *M. Tuberculosis* Complex Real-Time PCR Kit	**PCR**	100 reactions	MTBC

For Veterinary Use Only. For *In Vitro* Use Only.

Note: All products listed as PCR are qPCR products.

Ruminant—bovine/ovine/caprine (continued)

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Abortive/reproductive pathogens				
Bluetongue virus (BTV)	VetMAX BTV NS3 All Genotypes Kit	**PCR**	100 reactions	BTVNS3
Bovine herpesvirus type 4 (BHV-4)	VetMAX BHV Type 4 Kit	**PCR**	50 reactions	BHV450
Bovine viral diarrhea virus (BVDV)	VetMAX BVDV Screening Kit	**PCR**	100 reactions	B12S
	VetMAX-Gold BVDV PI Detection Kit	**PCR**	100 reactions	4413938
Coxiella burnetii	VetMAX *C. burnetii* Feces Kit	**PCR**	100 reactions	FQPE
Tritrichomonas foetus	VetMAX-Gold Trich Detection Kit	**PCR**	100 reactions	4483869
Bovine mastitis				
Mycoplasma bovis	VetMAX *M. bovis* Kit	**PCR**	50 reactions	MPBO50
Bovine tuberculosis				
Mycobacterium bovis	BOVIGAM *Mycobacterium bovis* Gamma Interferon Test System für Rinder (Germany)	**ELISA**	10 x 96-well plates; incl. 2 x PPD and 1 x PWM	7610910
Foot-and-mouth disease virus (FMDV)				
Foot-and-mouth disease virus (FMDV)	PrioCHECK FMDV NS Antibody ELISA Kit, strip plates	**ELISA**	5 x 96-well plates	7610770
	PrioCHECK FMDV NS Antibody ELISA Kit, solid plates (Brazil)	**ELISA**	5 x 96-well plates	7630120
	PrioCHECK FMDV NS Antibody ELISA Kit, solid	**ELISA**	10 x 96-well plates	7610760
		ELISA	5 x 96-well plates	7610440
	PrioCHECK FMDV NS STP Antibody ELISA Kit, strip plates (Brazil)	**ELISA**	5 x 96-well plates	7630130
	PrioCHECK FMDV Type A Antibody ELISA Kit, strip	**ELISA**	5 x 96-well plates	7610850
	PrioCHECK FMDV Type O Antibody ELISA Kit, strip	**ELISA**	5 x 96-well plates	7610420
	PrioCHECK FMDV Type Asia 1 Antibody ELISA Kit, strip	**ELISA**	5 x 96-well plates	7610870
	PrioCHECK FMDV NS Antibody ELISA Kit, strip plates (US)	**ELISA**	5 x 96-well plates	7630140

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Note: All products listed as PCR are qPCR products.

Ruminant—bovine/ovine/caprine (continued)

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Intestinal pathogens				
Coronavirus spp., rotavirus	VetMAX Ruminant Rotavirus & Coronavirus Kit	**PCR**	50 reactions	RRC50
Mycobacterium avium subsp. *paratuberculosis* (MAP)—Johne's disease	PrioCHECK MAP Ab 2.0 Plate Kit	**ELISA**	5 x 96-well plates	63325
	VetMAX *M. paratuberculosis* 2.0 Kit	**PCR**	100 reactions	MPTSA
	VetMAX MAP IS900-F57 Kit	**PCR**	100 reactions	TMPT
Mycobacterium avium subsp. *paratuberculosis* (MAP)—Johne's disease	VetMAX-Gold MAP Detection Kit	**PCR**	100 reactions	A29809
Pasteurella multocida	VetMAX *P. multocida* Toxinogenic Kit		50 reactions	PMTP50
Respiratory pathogens				
Bovine respiratory syncytial virus (BRSV)	VetMAX BRSV PI3 Kit	**PCR**	50 reactions	TRSVPI350
Coronavirus spp., *H. somni, M. bovis, M. haemolytica, P. multocida*, PI3, respiratory syncytial virus (RSV)	VetMAX Ruminant Respiratory Screening Kit	**PCR**	25 reactions	SRPR
Other pathogens				
Caprine arthritis encephalitis virus (CAEV)	PrioCHECK MAEDI-VISNA & CAEV Ab Serum Kit (France)	**ELISA**	5 x 96-well plates	VETCAEV5
Epizootic hemorrhagic disease virus (EHDV)	VetMAX EHDV Kit	**PCR**	50 reactions	EHDV50
Mycoplasma agalactiae and *M. mycoides*	VetMAX *M. agalactiae* & *M. mycoides* Kit	**PCR**	50 reactions	TMYCAS50
Peste des petits ruminants virus	VetMAX Peste des Petits Ruminants Virus Kit	**PCR**	50 reactions	PPRP50

Porcine

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Aujeszky's disease or pseudorabies				
Pseudorabies virus gB and gE	PrioCHECK PRV gE 2.0 Antibody ELISA Kit	**ELISA**	5 x 96-well plates	7589010
	PrioCHECK PRV gB Antibody ELISA Kit	**ELISA**	5 x 96-well plates	7589100
	PrioCHECK PRV gB Antibody ELISA Kit (Brazil)	**ELISA**	5 x 96-well plates	7589130
Classical swine fever				
Classical swine fever virus (CSFV)	PrioCHECK CSFV Ab 2.0 Kit (Brazil)	**ELISA**	5 x 96-well plates	7630100
	PrioCHECK CSFV Ab ELISA Kit, solid	**ELISA**	5 x 96-well plates	7610046
	PrioCHECK CSFV ERNS Ab Kit, strip	**ELISA**	5 x 96-well plates	7610370
	PrioCHECK Porcine CSFV Ab 2.0 Kit, strip	**ELISA**	5 x 96-well plates	7610600

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Note: All products listed as PCR are qPCR products.

Porcine (continued)

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Foot-and-mouth disease virus (FMDV)				
Foot-and-mouth disease virus (FMDV)	PrioCHECK FMDV NS Antibody ELISA Kit, strip plates	**ELISA**	5 x 96-well plates	7610770
	PrioCHECK FMDV NS 5PL SOL (450 samples) solid (Brazil)	**ELISA**	5 x 96-well plates	7630120
	PrioCHECK FMDV NS Antibody ELISA Kit, solid	**ELISA**	10 x 96-well plates	7610760
		ELISA	5 x 96-well plates	7610440
	PrioCHECK FMDV Type A Kit (450 sample strips)	**ELISA**	5 x 96-well plates	7610850
	PrioCHECK FMDV Type O Antibody ELISA Kit, strip	**ELISA**	5 x 96-well plates	7610420
	PrioCHECK FMDV Type Asia 1 (450 sample strips)	**ELISA**	5 x 96-well plates	7610870
	PrioCHECK FMDV NS STP (450 sample strips) (Brazil)	**ELISA**	5 x 96-well plates	7630130
	PrioCHECK FMDV NS 5PL (450 sample strips) (US)	**ELISA**	5 x 96-well plates	7630140
African swine fever virus (ASFV)				
African swine fever virus (ASFV)	PrioCHECK African Swine Fever Virus AB Kit	**ELISA**	5 x 96-well plates	A56981
	VetMAX African Swine Fever Virus Detection Kit	**PCR**	100 reactions	A28809
	VetMAX African Swine Fever Virus Detection Kit 2.0	**PCR**	100 reactions	A57008
Intestinal pathogens				
Brachyspira hyodysenteriae	VetMAX *B. hyodysenteriae* Kit	**PCR**	50 reactions	BHYO50
Meat harvest pathogens				
Mycobacterium avium subsp. *avium*	PrioCHECK Porcine M. *avium* Plate Kit, solid	**ELISA**	5 x 96 well plates	7610720
Salmonella serogroups B, C1, and D	PrioCHECK Porcine Salmonella Ab 2.0 Plate Kit	**ELISA**	50 x 96-well plates	7610670
	PrioCHECK Porcine Salmonella Ab 2.0 Strip Kit	**ELISA**	5 x 96-well plates	7610660
	PrioCHECK Porcine Salmonella Kit	**ELISA**	4,500 samples	7610480
Toxoplasma gondii	PrioCHECK Porcine Toxoplasma Ab Kit strip plates	**ELISA**	5 x 96-well plates	7610230
Trichinella spp.	PrioCHECK Porcine Trichinella Ab Strip Kit	**ELISA**	5 x 96-well plates	7610150
Respiratory pathogens				
Porcine circovirus type 2 (PCV2)	VetMAX Porcine PCV2 Quant Kit	**PCR**	100 reactions	QPCV
Porcine reproductive and respiratory syndrome virus (PRRSV), NA and EU strains	PrioCHECK Porcine PRRSV Ab strip Kit	**ELISA**	5 x 96-well plates	7610880
	VetMAX PRRSV EU & NA 2.0 Kit	**PCR**	100 reactions	A35751
Swine influenza virus (SIV) type A and genotypes	VetMAX Swine Influenza A-09 Kit	**PCR**	100 reactions	INFAPSWINE

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Note: All products listed as PCR are qPCR products.

Porcine (continued)

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Respiratory pathogens				
Porcine reproductive and respiratory syndrome virus (PRRSV), NA and EU strains	VetMAX PRRSV EU & NA 3.0 Kit	**PCR**	100 reactions	A57016
Swine influenza virus (SIV) type A and genotypes	VetMAX Swine Influenza A-09 Kit	**PCR**	100 reactions	INFAPSWINE
	VetMAX-Gold SIV Detection Kit	**PCR**	100 reactions	4415200
	VetMAX-Gold SIV Subtyping Kit	**PCR**	100 reactions	4485541
Other pathogens				
Swine vesicular disease virus (SVDV)	PrioCHECK Porcine SVDV Ab Kit (440 sample strips)	**ELISA**	5 x 96-well plates	7610205
Porcine parvovirus	VetMAX Porcine Parvovirus Kit	**PCR**	50 reactions	PPVP50

Avian

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Avian influenza type A	VetMAX-Gold AIV Detection Kit	**PCR**	100 reactions	4485261
Avian mycoplasmas (*Mycoplasma gallisepticum* and *Mycoplasma synoviae*)	VetMAX *M. gallisepticum* & *M. synoviae* Kit	**PCR**	100 reactions	MMAP
Newcastle disease virus (NDV)	VetMAX NDV Kit	**PCR**	100 reactions	NDV
Avian metapneumovirus	VetMAX Avian Metapneumovirus Kit	**PCR**	50 reactions	APVP50

Other species

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Anaplasma phagocytophilum	VetMAX *A. phagocytophilum* Kit	**PCR**	50 reactions	ANAP50
Chlamydophila spp.	VetMAX *Chlamydophila* spp. Exogenous IPC Kit	**PCR**	50 reactions	CHLPEXO50
Feline coronavirus (FCoV)	VetMAX FIP Dual IPC Kit	**PCR**	50 reactions	PIFP50
Mycobacterium tuberculosis	Primagam Non-Human Primate *M. tuberculosis* Interferon-Gamma Kit	**ELISA**	30 reactions	63311
Salmonella enterica spp.	VetMAX *S. enterica* spp. Kit	**PCR**	100 reactions	SALMSPP

For Veterinary Use Only. For *In Vitro* Use Only.

Note: All products listed as PCR are qPCR products.

Tuberculosis solutions

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Tuberculosis—skin test reagents				
Mycobacterium tuberculosis complex	Bovine Tuberculin PPD, 2 mL	**Skin test**	40-vial box (800 doses)	A49396
	Bovine Tuberculin PPD, 5 mL	**Skin test**	20-vial box (1,000 doses)	A49400
	Avian Tuberculin PPD, 2 mL	**Skin test**	40-vial box (800 doses)	A49432
	Avian Tuberculin PPD, 5 mL	**Skin test**	20-vial box (1,000 doses)	A49414
	Tuberculin PPD Kit, 2 mL (20 + 20 vials)	**Skin test**	20 avian and 20 bovine vials (400 doses each)	A49422
	Tuberculin PPD Kit, 5 mL (10 + 10 vials)	**Skin test**	10 avian and 10 bovine vials (500 doses each)	A49425

For Veterinary Use Only. For *In Vivo* Use Only. Prescription Only Medicine.

Note: All products listed as PCR are qPCR products.

Research use solutions

Our research use solutions are specifically designed for animal health laboratories seeking accurate and reliable data for their research and development endeavors.

Ruminant—bovine/ovine/caprine

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Research use only (RUO) products				
Bovine herpesvirus type 1 (BHV-1), infectious bovine rhinotracheitis (IBR)	VetMAX IBR/BHV-1 Reagents	**PCR**	100 reactions	4415203
Tritrichomonas foetus	*T. foetus* Reagents TAMUC	**PCR**	100 reactions	A51086
	T. foetus Controls TAMUC	**PCR**	100 reactions	A51087
Foot-and-mouth disease virus (FMDV)	Foot and Mouth Disease Reagent	**PCR**	100 reactions	4486952
	PrioCHECK FMD IPC Basic Kit	**ELISA**	1 kit	7610820

Porcine

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Intestinal pathogens				
Rotavirus A, Rotavirus B, Rotavirus C	VetMAX Rotavirus A/B/C Reagents	**PCR**	100 reactions	A57449
	VetMAX Rotavirus A/B/C Controls	**PCR**	100 reactions	A57451
Porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), pseudorabies virus (PRV-A)	VetMAX Swine Enteric Panel (TGEV, PEDV, PRV-A)	**PCR**	100 reactions	4486975
	VetMAX PEDV/TGEV Multiplex	**PCR**	100 reactions	4486979
Brachyspira hyodysenteriae	VetMAX B. HYO Reagents	**PCR**	100 reactions	A56661
	VetMAX B. HYO Controls	**PCR**	100 reactions	A56663
Foot-and-mouth disease virus (FMDV)				
Foot-and-mouth disease virus (FMDV)	PrioCHECK FMD IPC Basic Kit	**ELISA**	1 kit	7610820
African swine fever virus (ASFV)	DCA—Buffers CBTS (China)	**ELISA**	1 box	A54443
	DCA—Buffers PNS (China)	**ELISA**	1 box	A54444
	DCA—Test Plate (China)	**ELISA**	100 reactions	A54445
Meat harvest pathogens				
Trichinella spp.	PrioCHECK Trichinella AAD 10 ASY 1,000 individual animals	**Artificial digestion**	10 assays	7620030
	PrioCHECK Trichinella AAD Bulk 400,000 individual animals	**Artificial digestion**	4,000 assays	7620040
Other RUO products				
Classical swine fever virus (CSFV)	VetMAX Classical Swine Fever Reagents	**PCR**	100 reactions	4486946
Foot-and-mouth disease virus (FMDV)	Foot and Mouth Disease Reagent	**PCR**	100 reactions	4486952
Lawsonia intracellularis	VetMAX *L. intracellularis* Reagent	**PCR**	100 reactions	4486958
Mycoplasma hyopneumoniae	VetMAX *M. hyopneumoniae* Control	**PCR**	10 reactions	4415198
	VetMAX *M. hyopneumoniae* Reagents, Applied Biosystems Design	**PCR**	100 reactions	4415217
Porcine circovirus type 2 (PCV2)	VetMAX Porcine Circovirus-2 Reagent	**PCR**	100 reactions	4486961
Porcine reproductive and respiratory syndrome virus (PRRSV), NA and EU strains	VetMAX PRRSV 3.0 Reagents	**PCR**	100 reactions	A52576
	VetMAX PRRSV 3.0 Controls	**PCR**	10 reactions	A53170
	VetMAX PRRSV 3.0 MEX Reagents	**PCR**	100 reactions	A54571
	VetMAX PRRSV 3.0 MEX Controls	**PCR**	10 reactions	A54574

For Research Use Only. Not for use in diagnostic procedures.

Note: All products listed as PCR are qPCR products.

Research use solutions (continued)

Aquaculture

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Fish RUO products				
Infectious salmon anaemia virus (ISAV)	VetMAX Infectious Salmon Anaemia Virus (ISAV) Reagent	**PCR**	100 reactions	4486956
Shrimp RUO products				
IHHNV, white spot syndrome virus (WSSV)	VetMAX Shrimp IHHN & WSSV Reagent	**PCR**	100 reactions	4486969
Infectious myonecrosis virus (IMNV)	VetMAX Infectious Myonecrosis Virus Reagent	**PCR**	100 reactions	4486968
YHV, TSV, IMNV	VetMAX Shrimp YH, TS & IMN Reagent	**PCR**	100 reactions	4486970

Avian

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Research use only (RUO) products				
Avian mycoplasmas (*Mycoplasma gallisepticum* and *Mycoplasma synoviae*)	VetMAX *Mycoplasma Gallisepticum/Synoviae* Reagent	**PCR**	100 reactions	4486959
Newcastle disease virus (NDV)	VetMAX NDV Reagent	**PCR**	100 reactions	4406874
	VetMAX NDV and Xeno RNA Controls	**PCR**	1 kit	4406875

For Research Use Only. Not for use in diagnostic procedures.

Note: All products listed as PCR are qPCR products.

Food and environmental solutions

Our food and environmental testing solutions are designed to support the highest standards of safety and quality in animal health products.

Porcine

Ordering information

Pathogen/target	Product	Technology	Format	Cat. No.
Intestinal pathogens				
Porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), swine delta coronavirus (SDCoV)	VetMAX PEDV/TGEV/SDCoV Kit	**PCR**	100 reactions	A33402

For Testing of Food and Environmental Samples Only.

Note: All products listed as PCR are qPCR products.

Services

Our dedicated service team is ready to assist you with any inquiries or concerns regarding animal health solutions.

Customer support

Ordering information

Product	Duration	Cat. No.
1-Day On-Site Customer Training	1 day	A25784
1-Day Customer Training at Thermo Fisher Scientific	1 day	A25785
Implementation Program	4 days	A25786

ThermoFisher SCIENTIFIC

Learn more at **thermofisher.com/animalhealth**

applied biosystems

Regulatory requirements vary by country; products may not be available in your geographic area.

Certificate of Analysis

Certificat d'Analyse

applied biosystems
by Thermo Fisher Scientific

VetMAX™ NDV Kit

Σ	100 Tests	LOT	NDV-057
REF	NDV		2023-10-06
			2024-06-15

COMPONENT DESCRIPTION *Description du composant*	REF	UNIT	LOT
VetMAX™ NDV Reagents	4406874	1 Box	2304059
2X RT-PCR Buffer	8732G	1375 µL	2304152
25X RT-PCR Enzyme Mix	2737G	110 µL	2301134
25X NDV Primer Probe Mix	1020G	110 µL	2304057
Nuclease Free Water	9914G8	1750 µL	2212117
TaqMan™ NDV and Xeno™ RNA Controls	4406875	1 Box	2746725
25X NDV Control RNA	1023G	15 µL	2745538
Xeno RNA Control (10000 copies/µL)	5716G	110 µL	2688740
Nucleic Acid Dilution Solution	5717G	500 µL	2745976

INSTRUCTIONS FOR USE *Notices d'utilisation*

French	MAN0008825 RevB.0
English	MAN0008826 RevB.0

Quality Assurance

Laboratoire Service International
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69380 Lissieu - France
www.thermofisher.com/support

Date: 19 october 2023

VetMAX™ African Swine Fever Virus Detection Kit

TaqMan® real-time PCR detection of African swine fever virus

Catalog Number A28809

Doc. Part No. 100027918 Pub. No. MAN0010783 Rev. C.0

Technology	Species	Samples	Test type
Real-time PCR (DNA) • Duplex assay • Exogenous IPC	Swine	Blood Serum	Individual Pooled samples (5 or 10 samples)
		Tissues	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ VetMAX™ African Swine Fever Virus Detection Kit (Cat. No. A28809) enables detection of the African swine fever virus (ASFV) in swine blood, serum, or tissues by real-time PCR amplification of the ASFV P72 gene.

The assay is a single-well real-time PCR in which ASFV and exogenous Internal Positive Control (IPC) targets are amplified and detected using fluorescent TaqMan® probes.

The kit includes:

- 3 - Mix ASFV: Contains primers, TaqMan® probes, buffer, and enzyme for optimized duplex real-time PCR amplification of ASFV and IPC targets.
- 4a - EPC ASFV: Nucleic acid template for P72 target amplification. It serves as an external positive control for the real-time PCR reaction, and it is used to set the cycle threshold (C_t) for evaluating test results.
- 5 - IPC ASFV: Internal positive control added to each sample and control at the lysis step of the DNA extraction procedure. It serves as a control for the DNA purification process, and it is used to monitor for the presence of PCR inhibitors.

Contents and storage

Component	Amount[1]	Storage[2]
3 – Mix ASFV	2 × 1000 µL	–30°C to –10°C
4a – EPC ASFV	2 × 90 µL	
5 – IPC ASFV	1 × 500 µL	

[1] Sufficient for 100 25-µL real-time PCR reactions.
[2] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Applied Biosystems™ 7500 Real-Time PCR System	Contact your local sales office.
96-well plate, strip tubes (8- or 12-wells), microtubes or capillaries compatible with thermal cycler used	MLS
Nuclease-free pipettes and filtered pipette tips	MLS
Two ice buckets or refrigerated racks: • One for the PCR setup area where the PCR master mix is prepared • One for the area where DNA samples and controls are prepared	MLS
Plate covers or caps compatible with the plates, strip tubes, microtubes, or capillaries	MLS
Nuclease-free reagent tubes for preparing master mix	MLS
Nuclease-free Water (not DEPC-Treated)	AM9939
1X TE Buffer	MLS

Procedural guidelines

- For each real-time PCR run, include the controls indicated in “Set up the PCR reactions” on page 2.
- Follow “Good laboratory practices for PCR and RT-PCR” on page 4 to prevent false positives and contamination of test samples with PCR products.

Requirements for input DNA

We recommend using the MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359) for DNA extraction from biological samples, but you can also use other high quality DNA extraction methods after proper validation in your laboratory. In addition, prepare mock-purified sample using nuclease-free water as the starting material and the same DNA isolation method used for test samples.

IMPORTANT! Add 5 µL of 5 - IPC ASFV to the lysis solution used for DNA isolation for each sample and extraction control.

Before you begin

1. Thaw reagents and samples:
   a. Thaw 3 – Mix ASFV in an ice bucket or refrigerated rack.
   b. Thaw 4a – EPC ASFV, 5 – IPC ASFV, and DNA samples in a separate ice bucket or refrigerated rack.
2. Thoroughly mix the contents of each tube by vortexing, then briefly centrifuge.

Store thawed reagents, controls, and samples at 2–8°C until use.

Set up the PCR reactions

1. Dispense 20 µL of 3 – Mix ASFV to the appropriate number of PCR plate wells, strip tubes, or capillaries.
2. Add sample or control according to the following table:

Sample type	Component	Volume per reaction
Test sample	Sample DNA	5.0 µL
Positive control	4a – EPC ASFV	5.0 µL
Extraction control	Mock-purified sample	5.0 µL
No-template control (NTC)	Nuclease-free Water	5.0 µL

3. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
   - Reaction volume: 25 µL
   - Passive reference: ROX™ dye (included in 3 – Mix ASFV)

     Note: ROX™ dye must be set up if the instrument is capable of detecting it. Real-time PCR instruments that do not detect ROX™ dye can be used without affecting the accuracy of the reading.
   - Select detectors and assign TaqMan® probe reporter dyes and quenchers for each well, tube, or capillary used in the analysis.

Target	Reporter	Quencher
ASFV	FAM™ dye	Non-fluorescent quencher (NFQ)
IPC	VIC™ dye	TAMRA™ dye[1]

[1] TAMRA™ dye must be set up for real-time PCR analysis if the instrument is capable of detecting it. Real-time PCR instruments that do not detect TAMRA™ dye can be used without affecting the accuracy of the reading.

   - Thermal cycling program:

Stage	Repetitions	Temperature	Time
1	1	50°C	2 minutes
2	1	95°C	10 minutes
3	45	95°C	15 seconds
		60°C	1 minute

2. Run the thermal cycler program, collecting real-time amplification data during stage 3.

Guidelines for data analysis

- Follow the instrument user guide for raw data analysis.
- Set the thresholds for each target separately.
- Interpret the results for each control and sample according to the obtained C_t values as indicated in the following sections.

Validation criteria

Refer to the C_{tQC} values in the Certificate of Analysis for the manufacturing lot of the kit. The test is validated if the following criteria are met:

Reaction type	ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC}$ ASFV ± 3 C_t[1]	$C_t < 45$ or $C_t > 45$[2]	PCR is validated.
Extraction control[3]	$C_t > 45$	$C_t = C_{tQC}$ IPC ± 3 C_t[4]	DNA extraction is validated.
No-template control	$C_t > 45$	$C_t > 45$	PCR reagents are validated.

[1] See the EPC table in the Certificate of Analysis.
[2] The IPC value of the positive control is not used for test validation.
[3] Use the extraction control prepared using the same extraction procedure as the samples.
[4] See the IPC table in the Certificate of Analysis.

Interpretation of results

ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	ASFV is detected.
$C_t > 45$	$C_t = C_t$ of extraction control ± 3 C_t [1]	ASFV is not detected.
$C_t > 45$	C_t is outside this range: C_t of extraction control ± 3 C_t[1]	Invalid result.[2]

[1] The C_t value of the extraction control must first be validated as described in “Validation criteria” on page 3.
[2] The result is invalid due to a non-compliant IPC result.

Retest samples with invalid results

1. Dilute the DNA samples 1:10 in 1X TE buffer.
2. Repeat the real-time PCR procedure with 5 µL of the diluted DNA, then interpret the results as follows.

Result	Interpretation
The diluted DNA is positive for ASFV.	The result is validated.
The diluted DNA is negative for ASFV, and the IPC result is compliant.	
The diluted DNA is negative for ASFV, but the IPC result is non-compliant.	The result is invalid.

3. For diluted samples with invalid results, repeat the DNA isolation procedure on a new aliquot of the original sample lysate, then repeat the test.

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

World Organisation for Animal Health (OIE) Certification

Validated and certified by the OIE as fit for the purposes defined in the kit insert. Registration number: 20200114.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history: Pub. No. MAN0010783 (English)

Revision	Date	Description
C.0	21 July 2020	Added the World Organisation for Animal Health (OIE) logo and registration number.
B.0	8 February 2018	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Minor edits to align with current style.
A.0	2 April 2015	Baseline for revision history.

Development and validation of African Swine Fever Real-time PCR kit

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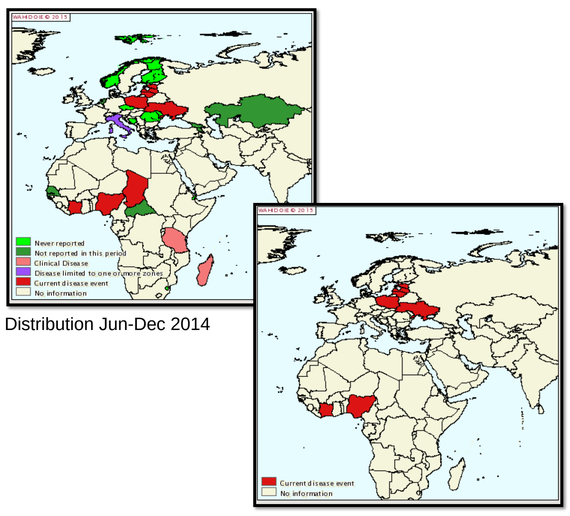
ABSTRACT

African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease that can cause significant economic losses. The disease is widely endemic in many parts of Africa, of Southern Europe and increasingly becoming a threat in Eastern Europe. As there is still no vaccine or treatment available, monitoring and controlling of the disease by means of diagnosis is the only way to control the disease and is of utmost importance. A new duplex real-time PCR kit that targets the p72 gene and an internal control has been developed and its performance for diagnosis of ASFV has been assessed. In order to demonstrate the sensitivity and specificity of the new LSI VetMAX™ African Swine Fever Virus detection kit, different internal and field studies including animal infection experiments were carried out (INIA, Spain; CVI, Netherlands; Germany). 1600 negative samples from ASFV free regions (Germany & Spain) and 33 different pathogens were tested to demonstrate specificity of the assay. About 100 ASFV positive samples from Africa and Europe were also tested. Results of the ASFV kit showed 100% sensitivity in all tested sample materials (blood, serum and tissues) and 100% specificity. No cross reaction was found with other pathogens and a serial dilution of the ASFV target sequence led to a limit of detection (LOD) of 16 genome copies per PCR reaction. The experimental LOD was 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood.
The LSI VetMAX™ African Swine Fever Virus detection kit fulfills all the validation criteria of PCR characteristics and complete method, as required by the NF U47-600-2 standard.

INTRODUCTION

African Swine Fever (ASF) is a DNA virus from the *Asfaviridae* Family. ASFV infects all *Suidae* (domestic and wild animals) but is not a human health threat. The virus is found in all body fluids and tissues of infected pigs. They usually become infected by direct contact with sick animals or by ingestion of infected products. ASFV is highly resistant in the environment. ASF disease is characterized by high fever, loss of appetite, haemorrhages in the skin and internal organs and death can occur within 2 to 10 days on average. ASF cannot be differentiated from classical swine fever by either clinical or post-mortem examination. It is an economically important disease that is widely endemic in many parts of Africa and that has become a real threat in Eastern Europe (Figure 1). In order to improve ASF diagnosis, a new duplex real time PCR kit was developed.

Figure 1. Disease Distribution maps - 2014 and 2015 (OIE)



Distribution Jun-Dec 2014

Distribution Jan-Mar 2015

MATERIALS AND METHODS

LSI VetMAX™ African Swine Fever Virus detection kit is a TaqMan™ ready-to-use real-time PCR assay based on the simultaneous detection of ASFV and an exogenous Internal Positive Control (IPC). For the development of a reliable, sensitive and specific rtPCR system, more than 450 different ASFV sequences representing the p72 protein encoding region were aligned. The isolation of viral DNA from field samples was performed with MagMax™ Pathogen RNA/DNA kit and MagVet™ Universal Isolation kit. About 1600 negative samples (blood and serum) were collected from ASFV free regions (Germany and Spain) and additionally 33 different pathogens close to ASFV or found in the same ecological niches were tested to demonstrate specificity of the assay. For validation of the sensitivity about 100 ASFV positive samples from Africa and Europe were tested. The limit of detection (LOD) was determined by serial dilution of a plasmid carrying a specific ASF sequence (pASF).

RESULTS

Table 1. Specificity of LSI VetMAX™ African Swine Fever Virus detection kit (partial data)

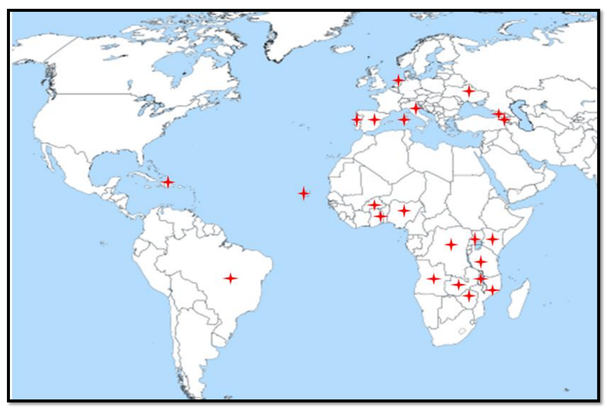
	Strain	ASFV Detection
Inclusivity	**ASFV (38 reference samples from CISA-INIA)**	Detected
	ASFV (20 strains from CVI)	Detected
Exclusivity	Classical Swine Fever Virus	Not detected
	Porcine circovirus 1	Not detected
	Porcine circovirus 2	Not detected
	Porcine Parvovirus	Not detected
	Herpes virus	Not detected
	PRRSV	Not detected
	Influenza H1N1	Not detected
	Mycoplasma hyopneumoniae	Not detected

The inclusivity of LSI VetMAX™ African Swine Fever Virus detection kit is evaluated on a panel of DNA isolated from 58 ASFV positive samples (organs and sera) coming from CISA-INIA, Spain and Central Veterinary Institute (CVI), Netherlands. As indicated in the table above, the kit show 100% inclusivity for the strains tested.

The exclusivity is assessed on a panel of 33 pathogens close to ASFV (data partially shown), either because they are preferentially found in the same ecological niches, phylogenetically close, or because they have the same clinical symptoms in target species. None of the strains tested is detected.

LSI VetMAX™ African Swine Fever Virus detection kit is specific for African Swine Fever Virus and does not detect other tested pathogens.

Figure 2. Distribution of strains tested for inclusivity



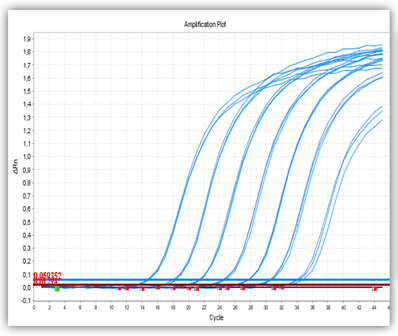
Strains and field samples tested (CISA-INIA, Spain and CVI, Netherlands) allow to recover a large distribution of the virus. All of them are detected by our PCR.

Table 2. Results obtained in ASFV positive strains (partial data)

Strain	ASFV Detection
Kat 67 - DRCongo	Detected
Malawi 82	Detected
Mozambique 64	Detected
Angola 72	Detected
Dominican Republic 80	Detected
Uganda 64	Detected
608 VR13	Detected
Lerida 1975 E75	Detected
Pontevedra 1970 E70	Detected
1207	Detected
BA71-V	Detected
L60 - Portugal	Detected
Haiti 78	Detected
Sassari 88	Detected
Dominican Republic 78	Detected
Lisbon 60	Detected
Georgia 2007	Detected
Spain - OURT 88/3	Detected
Tanzania KIRT 89/1	Detected
Zimbabwe VICT 90/1	Detected

A random set of 58 ASFV strains of different origins, including also the Georgia 2007 strain, which is representative for the ongoing outbreak in the Kaukasus, Russia, and neighbouring countries from 2007 to 2014 were tested.
All strains are detected and the results show a very high correlation between the Ct of LSI VetMAX™ African Swine Fever Virus detection kit and the in-house developed PCR in CVI or INIA (data not shown).

Figure 3. PCR efficiency of LSI VetMAX™ African Swine Fever Virus detection kit



R^2=0.998; Eff=101.5%

Table 3. Characteristics of LSI VetMAX™ African Swine Fever Virus detection kit according to AFNOR Standard for veterinary PCR (NF U47-600-2)

Characteristics	ASFV validation
Analytical specificity (Table 1)	100%
Efficiency (Figure 3)	Close to 100%
Limit of detection	16 copies per PCR
Repeatability	CV<3.01 %
Intermediate precision	CV<4.09 %
Robustness	Unaffected by all parameters tested
Experimental LOD - Serum	5x10^3 cp per mL
Experimental LOD - Blood	1x10^4 cp per mL

The PCR efficiency of LSI VetMAX™ African Swine Fever Virus detection kit, assessed from serial dilutions of a quantified ASF plasmid (pASF) until signal extinction, tested in triplicate, is close to 100% (Figure 3).

The limit of detection (LOD), evaluated on a quantified ASF plasmid, is estimated to be 16 copies of nucleic acids per PCR.

Repeatability and intermediate precision are evaluated at: coefficients of variation less than 4.09%.

For robustness, variations of temperature of hybridization (59° C, 60° C and 61° C), time of hybridization (54 sec, 60 sec and 66 sec), mix volume (18µL, 20µL and 22µL) and nucleic acid volume (4.5µL, 5µL and 5.5µL) do not affect the ASF PCR.

To determine the experimental limit of detection, serial dilutions of quantified plasmid are prepared to spike negative matrix at different concentration levels. The detection limit of MagMax™ Pathogen RNA/DNA method was estimated at 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood in individual samples.

Pooled assays are also performed by evaluating one positive sample among 5 or 10 samples. Serial dilutions of quantified plasmid are prepared to spike negative matrix as for individual tests. Then this positive sample is diluted in 4 or 9 negative samples to mimic pooled samples. The results of experimental LOD obtained show the same results as when tested individually (Table 4).

Table 4. Results obtained for the experimental limit of detection

	MagMAX™ Pathogen RNA/DNA Kit		
Matrices	**Individual analysis**	**Analysis of pool of 5**	**Analysis of pool of 10**
Serum	5x10^3 cp/mL	5x10^3 cp/mL	5x10^3 cp/mL
Blood	1x10^4 cp/mL	1x10^4 cp/mL	1x10^4 cp/mL

Pooled tests show the same experimental limits of detection (LOD) than individual tests. Furthermore this method enables to increase the analysis capacity in labs during outbreaks and reduce cost per analysis.

LSI VetMAX™ African Swine Fever Virus detection kit fulfills the validation criteria of PCR characteristics and complete method required by the NF U47-600-2 standard.

Table 5. Results obtained from field studies

5.1. Blood & Serum

Blood, Serum		Other methods		
		Positive	**Negative**	**Total**
LSI VetMAX™ African Swine Fever Virus detection kit	**Positive**	21	0	21
	Negative	0	1542	1542
	Total	21	1542	1563

The results show a correlation of 100% between both methods on positive blood and serum assays and show diagnostic specificity at: **Sp = 1542 / (1542+0) = 100%.**

5.2. Tissues

Tissues		Other methods		
		Positive	**Negative**	**Total**
LSI VetMAX™ African Swine Fever Virus detection kit	**Positive**	51	0	51
	Negative	0	6	6
	Total	51	6	57

The results show a correlation of 100% between both methods on negative tissues assays and show sensitivity at: **Se = 51 / (51+0) = 100%.**

For field studies, 1620 samples from various origins were tested. These field samples included various matrices (serum, blood and organs) at different levels of viral load (negative, low, medium, and high positive samples). 45 samples identified as positive or negative for ASF coming from a European Union Reference lab for ASFV (CISA-INIA, Valdeolmos, Spain); 1140 sera collected in a German slaughterhouse, a region free of ASFV; 400 blood samples collected on young pigs from weaning herds by a Spanish company, a region free for ASFV; and 36 samples from animal experiments carried out with 3 different ASFV strains from Central Veterinary Institute (CVI, Netherlands) were all evaluated.

Overall, in this assay, LSI VetMAX™ African Swine Fever Virus detection kit shows a diagnostic sensitivity of 100% on tissues and diagnostic specificity of 100% on blood and serum.

CONCLUSIONS

LSI VetMAX™ African Swine Fever Virus detection kit is a real-time PCR kit allowing the simultaneous detection of ASFV and an exogenous positive control in blood, serum and tissues samples.

The kit fulfills all the validation criteria for PCR characteristics and complete method required by the French standard (NF U47-600-2) "Requirements and recommandations for the development and validation of qRT-PCR in Animal Health".

The specificity, evaluated on different strains showed no cross-reactions with closely related pathogens. This kit had an efficiency close to 100% and its PCR limit of detection was16 copies per PCR (95% confidence interval).
The experimental LOD was 5x10^3 copies per mL in serum and 1x10^4 copies per mL in blood regardless of the test (individual or pool assays).
Test results on about 100 positive ASFV samples/strains and about 1600 negative samples showed 100% sensitivity on tissues and 100% specificity on blood and serum.

ASFV has significant economic impact and high mortality rate. The recent outbreaks of ASFV close to EU borders calls for a sensitive, reliable and specific real-time PCR such as the one described in this work.
LSI VetMAX™ African Swine Fever Virus detection kit provides a useful tool for an early detection of the ASF virus in various matrices from pigs and wild boars in order to guarantee the free status of pigs for trade. It helps enable control of the spread of disease and monitors circulating virus following outbreaks.

REFERENCES

▪ NF U 47-600-2 – Animal health analysis methods – PCR- Part 2: Requirements and recommandations for the development and the validation of veterinary PCR. (http://www.afnor.org)

ACKNOWLEDGEMENTS

▪ CISA-INIA, Valdeolmos, Spain
▪ CVI, Netherlands

TRADEMARKS/LICENSING

▪ LSI VetMAX™ African Swine Fever Virus detection kit (Cat. no. A28809)
▪ MagMax™ Pathogen RNA/DNA kit (Cat. No. 4462359)
▪ MagVet™ Universal Isolation kit (Cat. No. MV384)

Thermo Fisher SCIENTIFIC

Certificate of Analysis

Certificat d'Analyse

applied biosystems
by Thermo Fisher Scientific

VetMAX™ African Swine Fever Virus Detection Kit

Σ	**100 Tests**	LOT	**ASFV-071**
REF	**A28809**		**2023-11-30**
			2024-11-27

KIT CONFIGURATION *CONFIGURATION DU KIT*

COMPONENT DESCRIPTION *Description du composant*	REF	UNIT	LOT	
3 - Mix ASFV	MPEASFV	2 x 1000 µL	MPEASFV-071	2024-11-27
4a - EPC ASFV	EPCASFV	2 x 90 µL	EPCASFV-068	2024-11-29
5 - IPC ASFV	IPCASFV	500 µL	IPCASFV-055	2024-11-30

INSTRUCTIONS FOR USE *Notices d'utilisation*

English	MAN0010783 RevC.0
Spanish	MAN0018204 RevB.0
French	MAN0019541 RevA.0

QUALITY CONTROL RESULTS *RESULTATS DE CONTROLE QUALITE*

EPC *EPC*	**Already extracted EPC** *EPC déjà extrait*	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
4a - EPC ASFV	$C_{t\ QC}$ ASFV = **25**	ABI 7500 (Applied Biosystems)

IPC *IPC*	**Magnetic beads extraction** *Extraction billes magnétiques*	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
5 - IPC ASFV	$C_{t\ QC}$ IPC = **28**	ABI 7500 (Applied Biosystems)

$C_{t\ QC}$: Indicative Ct of the Quality Control *C_t indicatif du Contrôle Qualité*

SUPPLEMENTAL INSTRUCTIONS - Available upon request to Eurotech@thermofisher.com
Notices d'utilisation complémentaires - Disponibles sur demande auprès de Eurotech@thermofisher.com

Amplification Protocol *Protocole d'amplification*		**Nucleic acid purification Protocol** *Protocole de purification d'acides nucléiques*
Polish	MAN0018346 RevB.0	**N/A**

L. GOUNON

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Date: 12 December 2023

Verification of the detection of African swine fever virus in food matrices with real-time PCR

Summary

In this report, we show that a diagnostic workflow that includes the Applied Biosystems™ VetMAX™ African Swine Fever Virus Detection Kit and the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit is suited for the detection of African swine fever virus (ASFV) in food matrices, including cooked sausage meat.

Test performance was compared with two World Organisation for Animal Health (OIE)-prescribed and widely used ASFV qPCR assays, the King assay [1] and the Universal Probe Library (UPL) assay [2]. In all instances, the VetMAX ASFV kit detected the virus with higher sensitivity.

Both manual and automated protocols for the MagMAX CORE kit gave good results. In a comparison of manual extraction methods, the MagMAX CORE kit provided lower C_t values than a DNA extraction kit from another supplier, indicating a higher yield of high-quality DNA.

Introduction

We conducted an assessment of a real-time PCR workflow for the detection of ASFV in food matrices, specifically sausage meat for human consumption. This workflow comprises the VetMAX ASFV Detection Kit and the MagMAX CORE Nucleic Acid Purification Kit and was originally developed to detect ASFV in porcine blood and tissues. The MagMAX CORE kit has been designed for the purification of nucleic acid from a range of veterinary sample types. The VetMAX ASFV kit is a single-well duplex qPCR assay designed to deliver quantitative results on the presence of the ASFV genome in DNA extracts. The evaluation of the workflow has been carried out using sausage meat seeded with ASFV-containing material, at virus concentrations representative of those likely to be found in food matrices. The evaluation was performed in comparison with two OIE-prescribed ASFV qPCR assays, the King assay and the UPL assay.

Results

Sensitive ASFV detection in meat samples

The results from the performance of the VetMAX ASFV Detection Kit alongside the King and UPL assays are shown in Table 1. The MagMAX CORE kit in combination with the VetMAX ASFV kit detected ASFV in all 4 spiked meat samples (A–D), with lower C_t values than those obtained with the King or UPL assays. The mean differences in C_t between the VetMAX ASFV kit and the King and UPL assays were –1.62 and –1.41, respectively. These are statistically significant using one-way ANOVA ($P < 0.044$). The mean C_t of the exogenous internal positive control (EIPC) for the VetMAX ASFV kit was 26.98 (range 26.40–27.82), which fulfills kit criteria.

Table 1. C_t values generated using 3 qPCR assays for test samples A–D.

	Mean C_t (range)			
	A	B	C	D
VetMAX ASFV Detection Kit	32.48 (32.43–32.52)	34.63 (34.13–34.90)	35.05 (34.59–35.37)	32.52 (32.17–32.73)
King assay	33.82 (33.34–34.26)	36.23 (35.24–36.71)	36.86 (35.86–37.43)	34.23 (33.64–34.74)
UPL assay	34.20 (34.19–34.21)	36.44 (35.41–37.01)	35.66 (35.09–36.45)	34.01 (33.78–34.25)

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One aliquot of each test sample was manually extracted in duplicate using the MagMAX CORE kit and another supplier's DNA extraction kit (supplier 1). The extracted DNA was analyzed using the VetMAX ASFV kit, King assay, and UPL assay. Results are presented in Table 2.

DNA extracted with the MagMAX CORE Kit was found to yield lower C_t values on average than the supplier 1 kit when using the VetMAX ASFV kit (mean difference: –0.54), King assay (mean difference: –1.69), or UPL assay (mean difference: –0.42). In all instances, the VetMAX ASFV kit yielded lower C_t values than the King or UPL assays, which were found to be significant using one-way ANOVA (P = 0.001, 0.023, 0.044, and 0.004 for test samples A–D, respectively). The mean EIPC C_t for the VetMAX ASFV kit was 27.15 (range 26.69–27.47), which fulfills kit criteria. For the VetMAX ASFV kit, mean C_t values of manually extracted DNA correlated well between the MagMAX CORE and supplier 1 kits (r = 0.985, P = 0.013). Comparing manual and automated extraction using the MagMAX CORE kit, good correlation was found for the VetMAX ASFV kit (r = 0.993, P = 0.007), King assay (r = 0.934, P = 0.066), and UPL assay (r = 0.959, P = 0.041).

Detection of ASFV in cooked food matrices

To determine whether the real-time PCR workflow could be used to detect ASFV in cooked food matrices comparable to those suitable for human consumption, an aliquot of each test sample was heated to represent the cooking process. Each 1 g aliquot was heated to 76°C for 15 min on a heating block, then homogenized and processed by manual extraction using the MagMAX CORE kit. The results from the three qPCR assays are shown in Table 3.

The VetMAX ASFV kit was able to detect ASFV in all cooked meat samples and yielded lower C_t values on average than the King or UPL assays; however, this difference was not found to be significant using one-way ANOVA (P = 0.34). The King assay did not detect ASFV in the heat-treated test sample C.

The C_t values obtained from the heat-treated aliquots were higher than those obtained from raw test samples for all three qPCR assays (–1.66, –1.67, and –1.44 for the VetMAX ASFV, King, and UPL assays, respectively). However, this difference was not found to be significant using a 2-sample t-test (P < 0.144, P = 0.322, and P = 0.130 for the VetMAX ASFV, King, and UPL assays, respectively).

Table 2. C_t values generated with manually extracted nucleic acid and each of the 3 qPCR assays.

		Mean C_t		
Test sample	**Extraction kit**	**VetMAX ASFV kit**	**King assay**	**UPL assay**
A	MagMAX CORE	33.35	34.08	34.82
	Supplier 1	33.57	35.90	36.15
B	MagMAX CORE	35.06	36.25	36.58
	Supplier 1	36.03	36.43	36.31
C	MagMAX CORE	35.98	36.90	36.00
	Supplier 1	35.04	38.95	35.93
D	MagMAX CORE	32.99	33.24	33.78
	Supplier 1	32.99	36.30	33.47

Table 3. C_t values obtained from cooked meat product using manual extraction.

	Mean C_t			
	A	**B**	**C**	**D**
VetMAX ASFV Detection Kit	35.85	37.12	36.80	35.25
King assay	36.24	36.84	Undetected	35.49
UPL assay	37.44	37.60	36.40	35.49

Undetected: Not detected using qPCR.

Higher PCR inhibition in cooked meat samples

The VetMAX ASFV kit includes an EIPC that allows for the assessment of PCR inhibitors and the exclusion of false-negative results. EIPC C_t values from automated and manual extraction of raw and cooked testing matrices using the MagMAX CORE kit are shown in Table 4.

Table 4. EIPC C_t values for the VetMAX ASFV kit used on raw and cooked meat samples.

	Raw		Cooked
	C_t, automated	C_t, manual	C_t, manual
Mean (±SD)	26.98 (±0.30)	27.15 (±0.21)	29.38 (±0.26)
Range	(26.40–27.82)	(26.69–27.47)	(28.87–29.98)

Evaluating cooked test samples, the mean C_t for the EIPC was 29.38 (range 28.87–29.98), which was significantly higher (one-way ANOVA, $P < 0.001$) than observed with raw test samples. This indicates that the cooking process introduced PCR inhibitors, which may explain the higher C_t values observed in general for all qPCR assays and the negative results from the King assay in particular.

Conclusions

In this study, we have evaluated the detection of representative levels of ASFV contamination in food matrices. In all testing matrices, each containing different levels of ASFV spiking, samples processed utilizing the entire workflow yielded detectable ASFV. The VetMAX ASFV kit showed complete agreement with the King and UPL assays, two OIE-prescribed and widely used ASFV qPCR assays. In all instances, the VetMAX ASFV kit yielded lower C_t values on average than the King or UPL assays.

In a comparison of manual extraction methods, the MagMAX CORE kit provided lower ASFV C_t values than the supplier 1 DNA purification kit. In addition, manual extraction with the MagMAX CORE kit was used to successfully detect ASFV in cooked sausage meat. The results of this evaluation indicate that the diagnostic workflow using the VetMAX ASFV and MagMAX CORE kits is suitable for detection of the ASFV genome in food matrices.

Materials and methods

Preparation of samples for evaluation

A pig that was experimentally infected with ASFV genotype II (Georgia 2007/1 isolate) was euthanized at 8 days postinfection, having reached the humane endpoint. This experimental infection study was performed within a high-containment animal isolation facility in the United Kingdom (UK) in accordance with all local and internationally recognized animal husbandry regulations. Three sample types were obtained from this animal: loin, leg bone, and loin meat juice.

In addition, an ASFV genotype II isolate obtained from Hong Kong (Ref-SKU: 016V-03743, available through the European Virus Archive – Global (EVAg) website (european-virus-archive.com) was used in the experiments.

Sausage meat (containing >72% pork meat) was purchased from a UK supermarket and was used as the testing matrix. Four different test samples (A–D) were prepared by spiking the matrix with ASFV-containing material. The samples were tested for the presence of ASFV using the King assay [1] to determine ASFV levels. The results are shown in Table 5.

To prepare test samples A–D, 9.5 g of sausage meat was added to a labeled 50 mL centrifuge tube to which 500 µL of spiking material was added. The material was mixed manually for 2 min, and then 1 g of the material was distributed equally to 10 labeled 15 mL centrifuge tubes. All tubes were stored at –80°C until processing.

From our experience involving previous testing of ASFV-contaminated food products, we know that ASFV is typically detected at C_t values ranging between 34 and 38. We therefore diluted the test samples using sterile PBS to obtain comparable ASFV levels matching the real-life situation in subsequent experiments.

Table 5. Test samples prepared for evaluation.

Test sample	Spike	C_t
A	ASFV Hong Kong	19.79
B	Loin meat	28.55
C	Meat juice	27.31
D	Bone marrow	20.62

Processing of meat samples—homogenization

Approximately 1 g of sausage meat was added to a sterile mortar in addition to a small quantity of sterile sand. The material was ground using a sterile pestle to produce a paste. 5 mL of sterile PBS was added, and the suspension was transferred to a 15 mL centrifuge tube. The suspension was centrifuged at 3,000 x *g* for 5 min, following which the supernatant was removed and stored at 4°C until DNA extraction.

Automated extraction of ASFV DNA using the MagMAX CORE kit

Supernatant (200 µL) from the homogenate was added to a 2 mL microcentrifuge tube, followed by 450 µL of lysis solution (prepared by mixing 450 µL of MagMAX CORE Lysis Solution and 5 µL of VetMAX ASFV Internal Positive Control). The tube was vortexed for 3 min to create the lysate, and 600 µL was added to the designated well of a deep-well plate containing 30 µL of bead/PK mix (20 µL of MagMAX CORE Magnetic Beads and 10 µL of MagMAX CORE Proteinase K). Binding solution (350 µL) was added, and the plate was transferred to a Thermo Scientific™ KingFisher™ Flex Purification System for automated extraction. The "non-heated" script was selected, and following completion of the program, ASFV DNA was eluted into 90 µL of elution buffer. DNA extracts were stored at –20°C until further analysis.

Manual extraction of ASFV DNA using the MagMAX CORE kit

Supernatant (200 µL) from the homogenate was added to a 2 mL microcentrifuge tube to which 450 µL of lysis solution was added. The tube was vortexed for 3 min to create the lysate, and 600 µL was added to a tube containing 30 µL of bead/PK mix as previously described for automated extraction. Binding solution (350 µL) was added, and tubes were placed on an orbital shaker for 10 min. The tubes were then placed in a magnetic stand for 1 min, following which the supernatant was aspirated and discarded. The beads were washed using the magnetic stand, with 500 µL of MagMAX CORE Wash Solution 1 followed by 500 µL of MagMAX CORE Wash Solution 2. The resulting magnetic bead pellet was dried for 5 min prior to eluting ASFV DNA in 90 µL of elution buffer. DNA extracts were stored at –20°C prior to further analysis.

Manual extraction of ASFV DNA using supplier 1 kit

Supernatant (140 µL) from the homogenate was added to a 2 mL microcentrifuge tube containing 560 µL of lysis buffer and was incubated at room temperature for 10 min. Absolute ethanol (560 µL) was added, and the tube was mixed by pulse vortexing for 15 seconds. Then, 630 µL of the mixture was added to a microspin column. The column was centrifuged at 6,000 x *g*, and the filtrate was discarded. The same process was repeated with the remaining 630 µL of lysate. Wash buffer 1 (500 µL) was added to the spin column, which was centrifuged at 6,000 x *g*. The filtrate was discarded, and the process repeated. Then 500 µL of wash buffer 2 was added to the spin column, which was centrifuged at 12,000 x *g* and the filtrate discarded. ASFV DNA was eluted into 50 µL of elution buffer, and DNA extracts were stored at –20°C prior to further analysis.

ASFV qPCR assays

Real-time PCR assays using the VetMAX ASFV kit were performed according to the instructions for use (Pub. No. MAN0010783). Briefly, 5 µL of extracted DNA was added to a well containing 20 µL of VetMAX ASFV qPCR master mix. Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, and then 45 cycles of PCR, with each cycle consisting of 95°C for 15 sec and 60°C for 1 min. Analysis was performed on the Applied Biosystems™ 7500 Fast Real-Time PCR System using fast ramp rates.

The King and UPL assays for the detection of the ASFV genome in porcine blood and tissues are accredited by the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17025 requirements and are referenced in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [3]. The King and UPL assays were performed in accordance with NVR-SOP-20 and NVR-SOP-29, respectively. For the King assay, 18 µL of reaction mix was prepared using 10 µL of Applied Biosystems™ Path-ID™ qPCR Master Mix, 400 nM forward and reverse primers, and 250 nM probe. For the UPL assay, 18 µL of reaction mix was prepared using 10 µL of Path-ID qPCR Master Mix, 400 nM forward and reverse primers, and 100 nM probe. Both assays were performed using 2 µL of DNA extract. Cycling conditions were as follows: 95°C for 10 min and then 45 cycles of PCR, with each cycle consisting of 95°C for 15 sec and 60°C for 1 min. Analysis was performed on the 7500 Fast Real-Time PCR System using fast ramp rates.

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Evaluation of ASFV detection system and statistical analysis

From each of the test samples A–D, aliquots 1–3 were extracted in duplicate using either the automated procedure with the MagMAX CORE kit and KingFisher Flex Purification System, the manual procedure with the MagMAX CORE kit, or the manual procedure with the supplier 1 kit. Each DNA extract was then analyzed in duplicate using the VetMAX ASFV kit, the King assay, or the UPL assay.

To assess whether the diagnostic workflow with the VetMAX ASFV kit can be used on processed food matrices, an aliquot of each test sample was heated at 76°C for 15 min on a heating block. These aliquots were manually extracted in duplicate, and DNA extracts were analyzed in duplicate using the VetMAX ASFV kit, the King assay, or the UPL assay. C_t values from all qPCR assays were then compared, and statistical analysis was performed using Minitab™ statistical software version 17 (Minitab Inc., PA, USA).

References

1. King DP, Reid SM, Hutchings GH et al. (2003) Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. *J Virol Methods* 107(1):53–61.
2. Fernandez-Pinero J, Gallardo C, Elizalde M et al. (2013) Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library. *Transbound Emerg Dis* 60(1):48–58.
3. OIE (2018) Chapter 3.8.1: African swine fever (infection with African swine fever virus). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,* 8th Edition.

Ordering information

Product	Quantity	Cat. No.
VetMAX African Swine Fever Virus Detection Kit	100 reactions	A28809
MagMAX CORE Nucleic Acid Purification Kit	100 reactions	A32700
	500 reactions	A32702
Path-ID qPCR Master Mix	100 reactions	4388643
	500 reactions	4388644
7500 Fast Real-Time PCR System, desktop	1 instrument	4351107
KingFisher Flex Purification System with 96 Deep-Well Head	1 instrument	5400630

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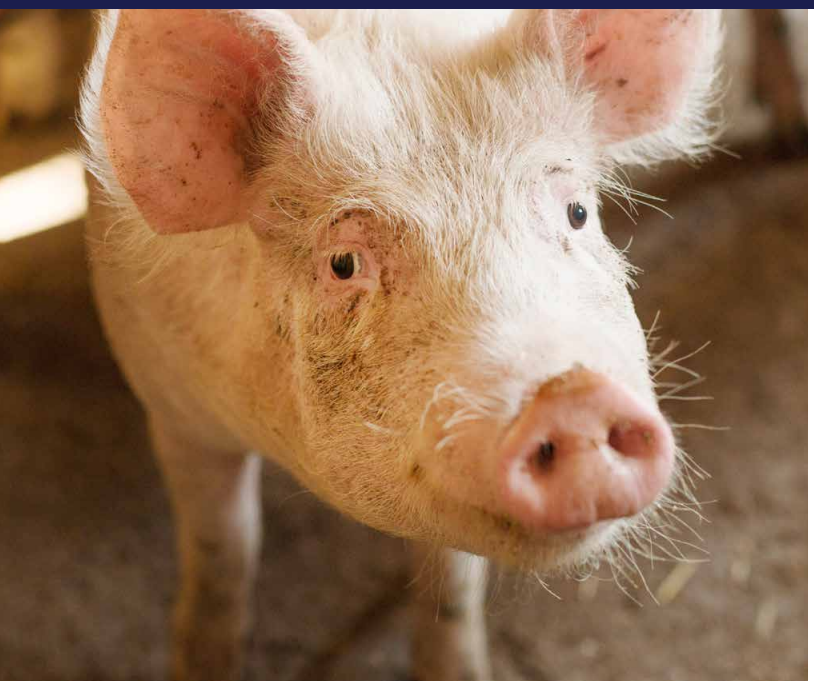

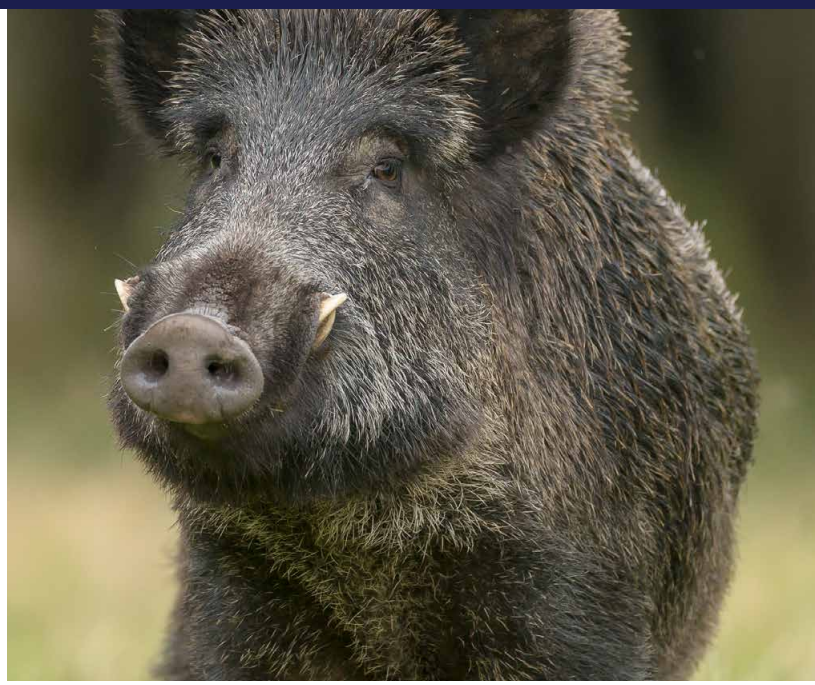

VetMAX African Swine Fever Virus Detection Kit

Real-time PCR kit—validated by the OIE* and used for virus circulation monitoring in outbreak situations

The Applied Biosystems™ VetMAX™ African Swine Fever Virus (ASFV) Detection Kit has been used since 2015 during outbreaks in Europe, Asia, and Africa for clinical confirmation and detection of ASFV in domestic and wild pigs.

Benefits

- Approved by the OIE for the detection of ASFV
- Validated by the European Union Reference Laboratory for ASFV (EURL, CISA-INIA, Spain)
- Detects all ASFV genotypes
- Allows users to test pools of up to 10 samples
- Contains a ready-to-use master mix for the detection of the ASFV target and the internal positive control (IPC)
- Delivers results in less than three hours

The VetMAX African Swine Fever Virus Detection Kit has successfully passed every step of the OIE procedure for the registration of diagnostic kits and has evidenced that it is fit-for-purpose for the detection of the African swine fever virus in blood, serum, and tissues of domestic and wild pigs (including wild boars).

Technology	Species	Samples	Test type
Real-time PCR of DNA • Duplex assay • Exogenous IPC	Domestic pig Wild boar	Blood Serum	Individual or pooled samples, up to 10
		Tissues	Individual

* World Organisation for Animal Health (OIE).

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Diagnostic sensitivity

The VetMAX African Swine Fever Virus Detection Kit has been evaluated by EURL on 424 positive samples coming from field genotype II ASFV-infected areas within Asian and European countries.

424 positive samples tested	400 positives using UPL-PCR* 94%	384 positives using the VetMAX African Swine Fever Virus Detection Kit 91%

Although the UPL-PCR test was able to detect the highest percentage of the infected animals, the kappa coefficient (κ) statistics were used to evaluate the concordance between each test, and the result of 0.87%$_{(95\%\ CI)}$ indicates **perfect agreement between the UPL reference method and the VetMAX African Swine Fever Virus Detection Kit.**

* Universal probe library (UPL) real-time PCR (Fernandez et al, 2013).

Inclusivity and exclusivity

PCR inclusivity has been evaluated on a panel of reference samples from CISA-INIA and CVI (Central Veterinary Institute, the Netherlands).

PCR exclusivity has been evaluated on various viruses, bacteria, and parasites.

	Strain	VetMAX African Swine Fever Virus Detection Kit
Inclusivity	38 reference samples from CISA-INIA	Detected
	20 reference samples from CVI	Detected
Exclusivity	Viruses (PCV1, PCV2, PPV, influenza, PRRSV, CSFV, BVDV, BHV1, porcine coronavirus, herpes virus type 1)	Not detected
	Bacteria (*Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae*, *Lawsonia* spp., *Brachyspira hyodysenteriae*, and 11 other species)	Not detected
	Parasites (*Toxoplasma gondii*, *Neospora caninum*)	Not detected

The VetMAX African Swine Fever Virus Detection Kit **correctly detected all ASF strains from CISA-INIA and CVI panels** and didn't show cross-reaction with other pathogens.

Real-time PCR workflow from sampling to result

Sample collection → **Nucleic acid extraction** → **Amplification**

Direct samples or those collected using Applied Biosystems™ GenoTube Livestock Swabs

36 min using the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit with a Thermo Scientific™ KingFisher™ instrument

90 min using the VetMAX ASFV Detection Kit with a PCR instrument such as the Applied Biosystems™ QuantStudio™ 5 system

Ordering information

Product	Quantity	Cat. No.
VetMAX African Swine Fever Virus Detection Kit	100 tests	A28809
Sample collection and sample preparation		
GenoTube Livestock Swab	1 tube	9062010
MagMAX CORE Nucleic Acid Purification Kit	500 tests	A32700

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Certificate of Analysis

Certificat d'Analyse

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by Thermo Fisher Scientific

VetMAX™ West Nile Virus Kit

Σ	50 Tests	LOT	WNPEXO-025
REF	WNPEXO50		2023-09-06
			2024-09-01

KIT CONFIGURATION *CONFIGURATION DU KIT*

COMPONENT DESCRIPTION *Description du composant*	REF	UNIT	LOT	
3 - Mix West Nile	MPEWN	2 x 500µL	MPEWN-025	2024-09-01
4a - EPC West Nile	EPCWN	90 µL	EPCWN-025	2024-09-06
5 - IPC West Nile	IPCWN	250 µL	IPCWN-025	2024-09-06

INSTRUCTIONS FOR USE *Notices d'utilisation*

French	MAN0008914 RevB.0
English	MAN0008915 RevB.0

QUALITY CONTROL RESULTS *RESULTATS DE CONTROLE QUALITE*

EPC *EPC*	**Already extracted EPC** *EPC déjà extrait*	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
4a - EPC West Nile	$C_{t\ QC}$ WN = **26**	ABI 7500 (Applied Biosystems)

IPC *IPC*	**Column extraction** *Extraction colonne*	**Thermocycler used for QC** *Thermocycleur utilisé pour le CQ*
5 - IPC West Nile	$C_{t\ QC}$ IPC = **28**	ABI 7500 (Applied Biosystems)
Endogenous IPC *IPC endogène*	$C_{t\ QC}$ IPC **< 40**	ABI 7500 (Applied Biosystems)

$C_{t\ QC}$: Indicative Ct of the Quality Control *C_t indicatif du Contrôle Qualité*

SUPPLEMENTAL INSTRUCTIONS - Available upon request to Eurotech@thermofisher.com
Notices d'utilisation complémentaires - Disponibles sur demande auprès de Eurotech@thermofisher.com

Amplification Protocol *Protocole d'amplification*	**Nucleic acid purification Protocol** *Protocole de purification d'acides nucléiques*
N/A	**French** MAN0008916 RevB.0

M. Mallen

Quality Assurance

Laboratoire Service International
6 allée des Ecureuils
Parc Tertiaire du Bois-Dieu
69380 Lissieu - France
www.thermofisher.com/support

Date: 20 September 2023.

VetMAX™ West Nile Virus Kit

TaqMan® real-time RT-PCR for detection of the West Nile virus

Catalog Number WNPEX050

Doc. Part No. 100020471 **Pub. No.** MAN0008915 **Rev.** B.0

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time RT-PCR (RNA) – Duplex – Endogenous/exogenous IPC	Horse	Whole blood, serum, plasma Cell culture supernatant Feces Tracheal and cloacal swabs Organs (cervix...) Cerebrospinal fluid	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Information about the product

Description of the product

The **Applied Biosystems™ VetMAX™ West Nile Virus Kit** is a molecular diagnostic tool for detecting West Nile virus by real-time RT-PCR.

Each RNA sample obtained after extraction is analyzed in a single well; the same well is used to specifically detect the viral RNA of West Nile virus and an IPC (Internal Positive Control). The kit simultaneously detects an endogenous IPC in cellular samples and an exogenous IPC to be added to non-cellular samples. A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

It can be used on viral RNA extracted from **whole blood, serum and plasma, cell culture supernatant, feces, tracheal and cloacal swabs, organs,** and **cerebrospinal fluid**.

Complete protocols for viral RNA extraction from these matrices are available upon request from Technical Support.

Kit contents and storage

The **VetMAX™ West Nile Virus Kit** contains components that can be used for detecting in duplex the West Nile virus and an IPC. Upon receipt, the whole kit must be stored at **–30°C to –10°C**. After initial use of a component, store it according to the following recommendations:

Component	Description	Volume (50 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix West Nile (Green tube)	Mix for TaqMan® RT-PCR. Contains: • The detection system for the West Nile virus target, including a TaqMan® probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher). • The detection system for IPCs, including several TaqMan® probes labeled **VIC™ – TAMRA™**. • Buffer, reverse transcriptase and real-time PCR enzyme.	2 × 500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC West Nile (Brown tube)	**External Positive Control:** Positive control for West Nile virus It consists of **already extracted** nucleic acid to be amplified during real-time RT-PCR.	90 µL	–30°C to –10°C	–30°C to –10°C
5 - IPC West Nile (Yellow tube)	**Internal Positive Control:** Exogenous internal control **to be added to each non-cellular and feces sample and each control** in the lysis step of the extraction.	250 µL	–30°C to –10°C	–30°C to –10°C

NOTE: For small extraction series, it is recommended that the IPC West Nile be aliquoted to avoid more than 3 cycles of freezing/thawing (a minimum volume of 50 µL).

Extraction and amplification controls

The **VetMAX™ West Nile Virus Kit** contains two controls, enabling validation of the extraction and the amplification of the viral RNA:

4a - EPC West Nile: positive control for West Nile

A positive control, **already extracted,** for amplification during the real-time RT-PCR.

A positive result within the specified C_t range validates the amplification of the West Nile target by real-time RT-PCR.

5 - IPC West Nile: extraction internal control (optional use depending on the type of sample)

Positive control **to be added to each non-cellular and feces sample during the lysis step** of the nucleic acid extraction. For cellular samples, nucleic acid extraction validation is performed using an endogenous IPC present in each cellular sample.

A positive IPC result with a compliant value in a cellular sample (for an endogenous IPC) or within the specified C_t range in a sample validates the extraction of this non-cellular or feces sample (for an exogenous IPC), whether positive or negative for the target pathogen, thus eliminating false negatives and verifying the effect of the inhibitors.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of components used in the extraction without addition of the sample (sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water) that undergoes the same treatment as the samples: nucleic acid extraction (with or without IPC added) and real-time RT-PCR.

A negative result for the West Nile Virus and the endogenous IPC (for cellular samples) confirms the absence of contamination during the extraction and the real-time RT-PCR.

NC: negative amplification control

This control consists of an amplification mix added to the plate during real-time RT-PCR preparation, as well as 5 µL of DNase/RNase-free water to adjust the reaction to 25 µL.

A negative result for the West Nile virus and the IPC confirms the absence of contamination during real-time RT-PCR reaction preparation.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

Analysis procedure

The real-time PCR reaction volume is 25 µL:

- **3 - Mix West Nile**: 20 µL per analysis
- **Extracted RNA:** 5 µL per analysis

Extraction of viral RNA

RNA must be extracted from the samples for real-time RT-PCR analysis.

For non-cellular and feces samples, add **5 µL** of **5 - IPC West Nile** to each sample to be extracted and the NCS in the lysis step of the nucleic acid extraction.

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ West Nile Virus Kit, please contact Technical Support.

Preparation of the real-time RT-PCR

1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Thaw the tube of **3 - Mix West Nile** at **2°C to 8°C on ice** or on a refrigerated rack.
3. Mix the tube of **3 - Mix West Nile** by shaking gently, then centrifuge briefly.
4. Add **20 µL of 3 - Mix West Nile** to each PCR plate well, PCR strip or capillary used.
5. Add RNA from the samples and controls to the reaction mix, according to the pre-defined analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	RNA extracted from sample	5 µL
Positive amplification control	**4a - EPC West Nile**	5 µL
Negative lysis control (NCS)	Extracted NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

Amplification by real time RT-PCR

1. Create the following detectors on the thermal cycler:

	Reporter	Quencher
WN	FAM™	NFQ (Non-Fluorescent Quencher)
IPC WN	VIC™	TAMRA™[1]
Passive reference: ROX™[1]		

[1] The fluorophores TAMRA™ and ROX™ are required for real-time RT-PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, the absence of detection of these fluorophores does not affect the real-time RT-PCR analysis.

2. Assign the **WN** detector and the **IPC WN** detector to each sample well used in the analysis.
3. Set up the following real-time RT-PCR program for the analysis:

	Step repetitions	Temperature	Duration
Step 1	×1	45°C	10 minutes
Step 2	×1	95°C	10 minutes
Step 3	×40	95°C	15 seconds
		65°C[1]	45 seconds

[1] Collection of fluorescence data during the 65°C – 45 seconds stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time RT-PCR.

Analysis of the results

Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time RT-PCR.
2. For each detector, interpret the results according to the sample C_t values obtained as recommended below.

Validation

The test is validated if the following criteria are met:

	West Nile detector	West Nile IPC detector	Validation
EPC West Nile	$C_t = C_{t\,QC}$ West Nile of **4a - EPC West Nile** ± $3C_t$[1]	For the endogenous IPC: $C_t < 40$ or $C_t > 40$[2]	PCR validated
NCS	$C_t > 40$	If endogenous IPC (without exogenous IPC added): $C_t > 40$	Extraction validated
		If exogenous IPC added: $C_t = C_{t\,QC}$ IPC of **5 - IPC West Nile** ± $3C_t$[3]	
NC	$C_t > 40$	$C_t > 40$	PCR components validated

[1] Refer to the values listed in section 2.1 "EPC" of the Certificate of Analysis of the lot used for the test.
[2] The IPC value in the EPC should not be used for test validation.
[3] Refer to the values listed in section 2.2 "IPC" of the Certificate of Analysis of the lot used for the test.

Interpretation of results

For each sample analyzed, the results should be interpreted as shown below:

For non-cellular and feces samples:

West Nile detector	West Nile IPC detector (exogenous IPC)	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	West Nile virus detected
$C_t > 40$	$C_t \leqslant C_t$ IPC of NCS + $3C_t$[1]	West Nile virus not detected
$C_t > 40$	$C_t > C_t$ IPC of NCS + $3C_t$[1]	Not validated[2]

[1] Refer to the IPC C_t value obtained for the NCS done during the same extraction series as the samples to be analyzed. The IPC C_t value obtained for this NCS must first be validated as described above.
[2] The sample will be returned as not validated due to the negative IPC.

For cellular samples:

West Nile detector	West Nile IPC detector (endogenous IPC)	Interpretation
$C_t < 40$	$C_t < 40$ or $C_t > 40$	West Nile virus detected
$C_t > 40$	$C_t < 40$	West Nile virus not detected
$C_t > 40$	$C_t > 40$	Not validated[1]

[1] The sample will be returned as not validated due to the negative IPC.

Procedure for handling non-validated samples

1. Dilute the RNA at a 1:10 dilution in 1X TE buffer.
2. Denature the diluted RNA.
3. Perform a new RT-PCR analysis on 5 µL of this dilution (after denaturation).
4. If the diluted RNA is positive for West Nile with an acceptable IPC result, the result obtained is then validated.
5. If the diluted RNA is negative for West Nile with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the sample pre-diluted 1:10 in 1X PBS buffer before extraction.
6. If the result is still not validated, repeat the analysis on a new sample.

Documentation and support

Customer and technical support

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- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Translated from the French Pub. No. MAN0008914 Rev. B.0.

The information in this guide is subject to change without notice.

Revision history of Pub. No. MAN0008915 (English)

Revision	Date	Description
B.0	28 June 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	31 March 2014	Baseline for revision history