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

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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

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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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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Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**

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.

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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 %.

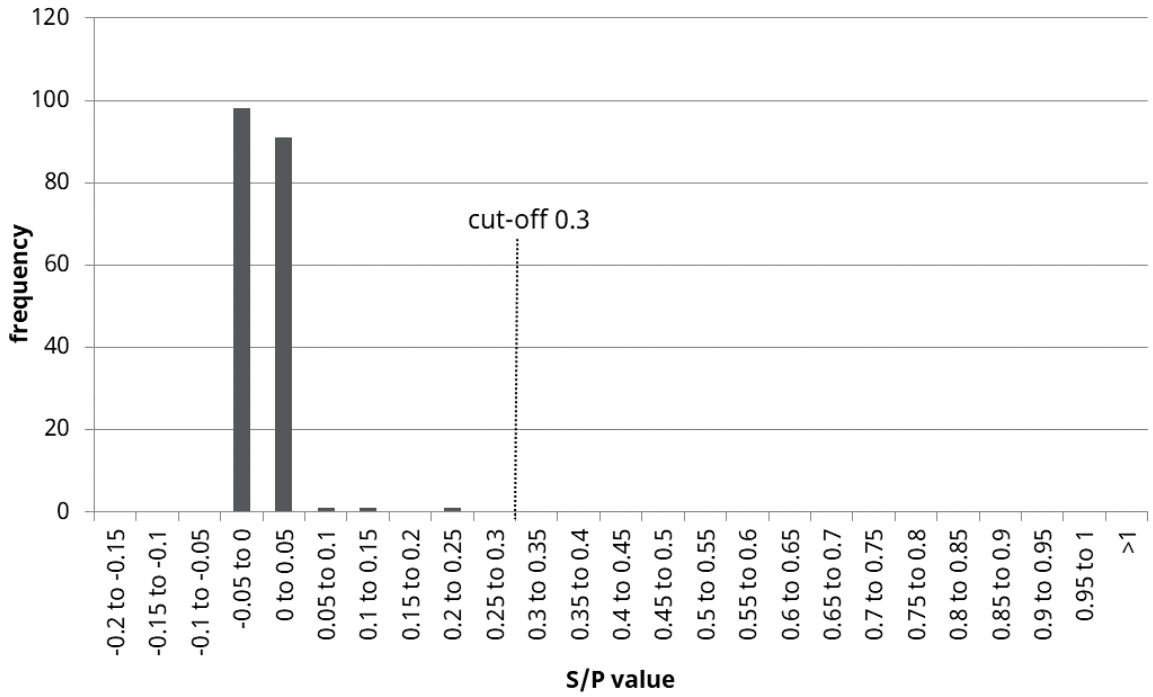

Figure 1. Frequency distribution of the S/P values of *n* = 192 AIV-antibody negative samples from SPF animals or control animals in a vaccination trial, tested with the flocktype AIV Ab

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).

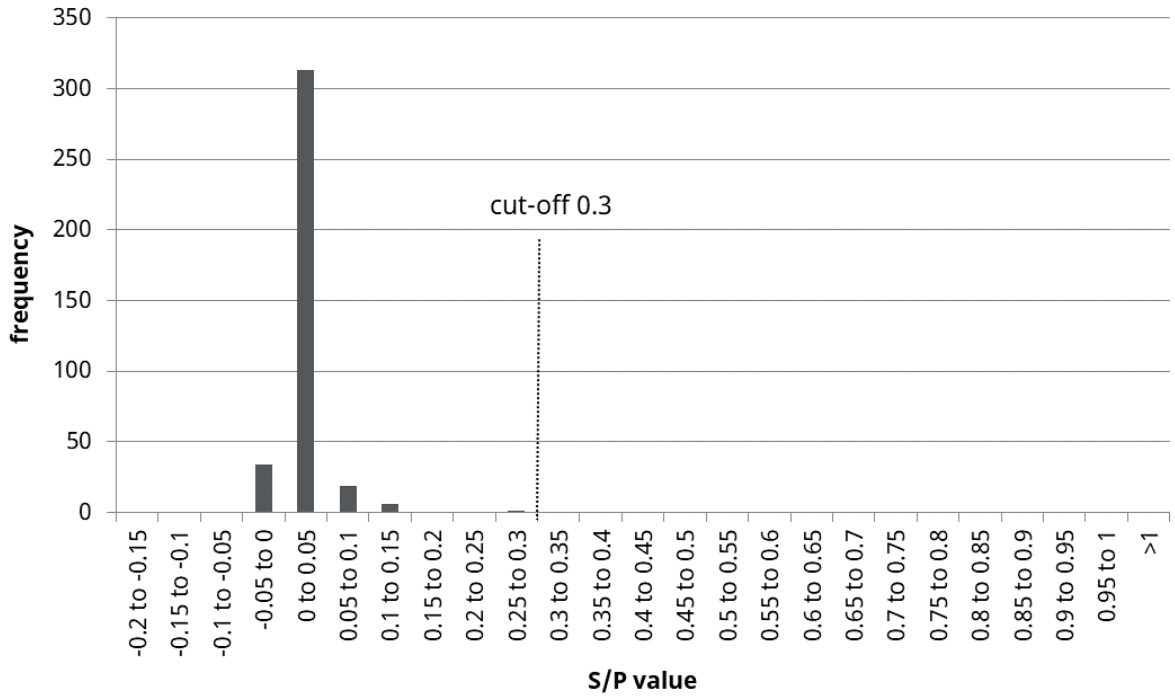

Figure 2. Frequency distribution of the S/P values of *n* = 371 AIV-antibody negative field samples tested with the flocktype AIV Ab

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.

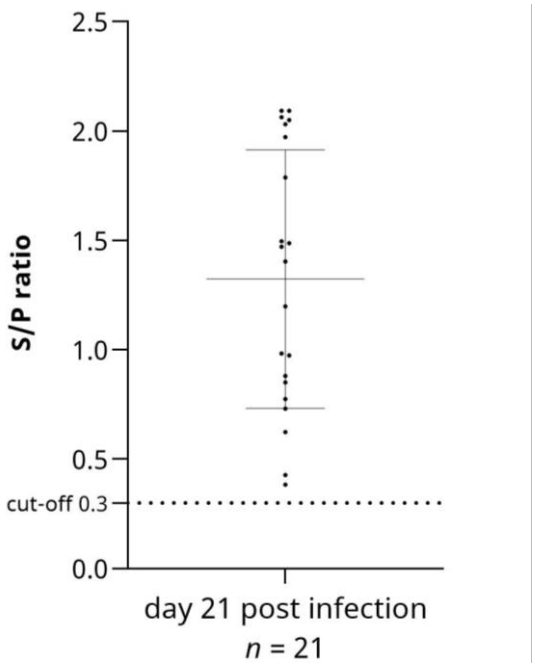

Figure 3. Results of the H5N2 infection study samples tested with the flocktype AIV Ab (bar: Mean with standard deviation)

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.

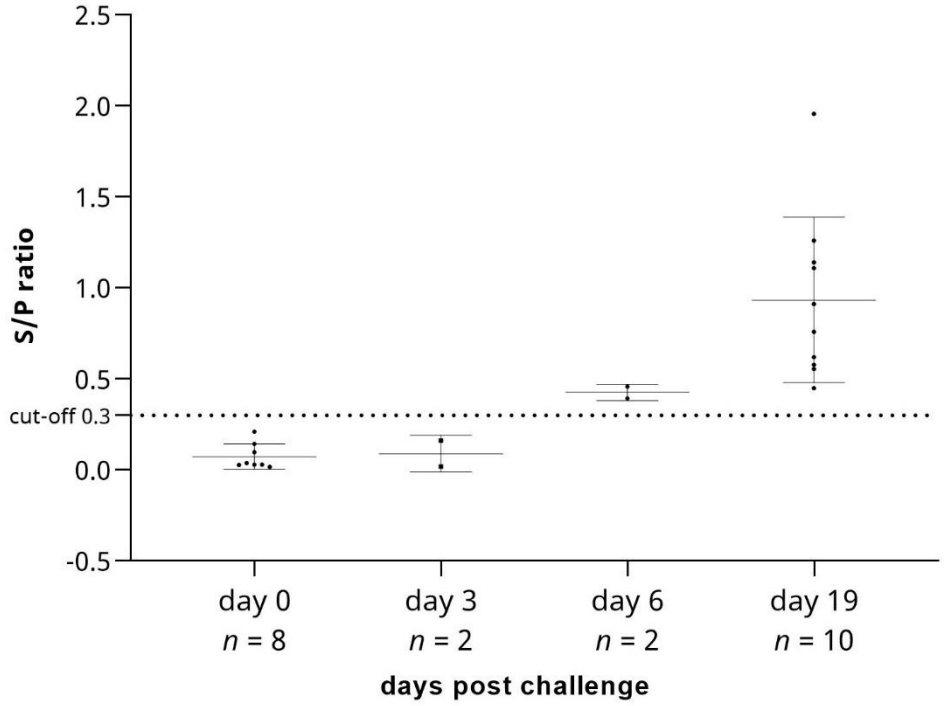

Figure 4. Results of the H7N1 vaccination and challenge study samples tested with the flocktype AIV Ab (bar: Mean with standard deviation)

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.

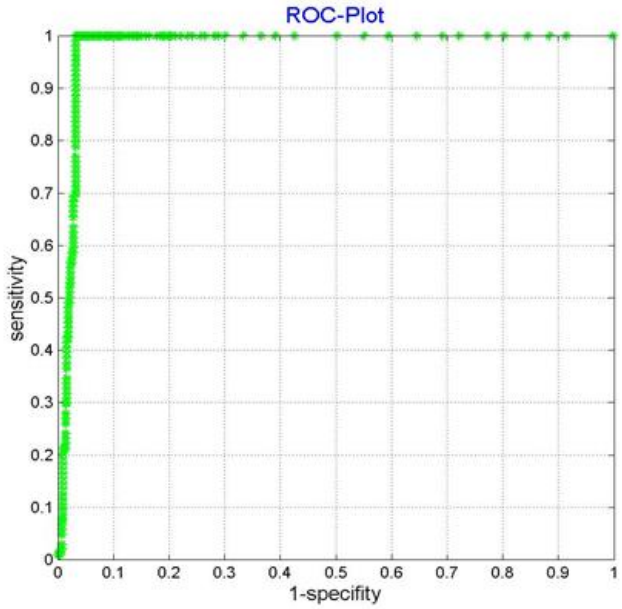

Figure 5. ROC plot for flocktype AIV Ab

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).

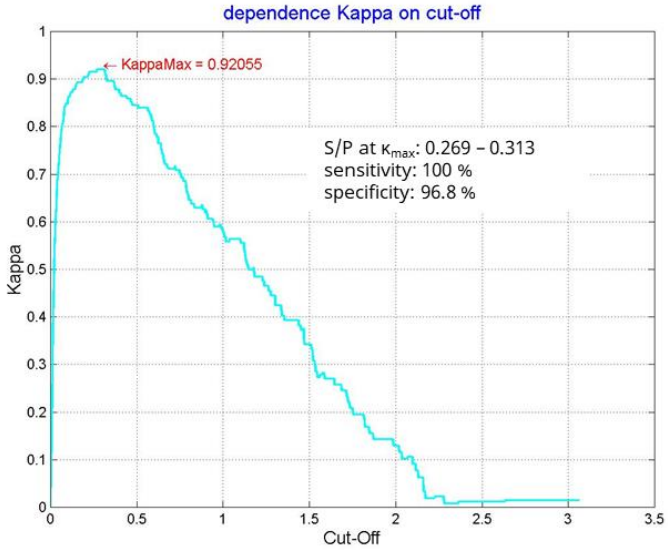

Figure 6. Dependence of κ on cut-off for flocktype AIV Ab

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.

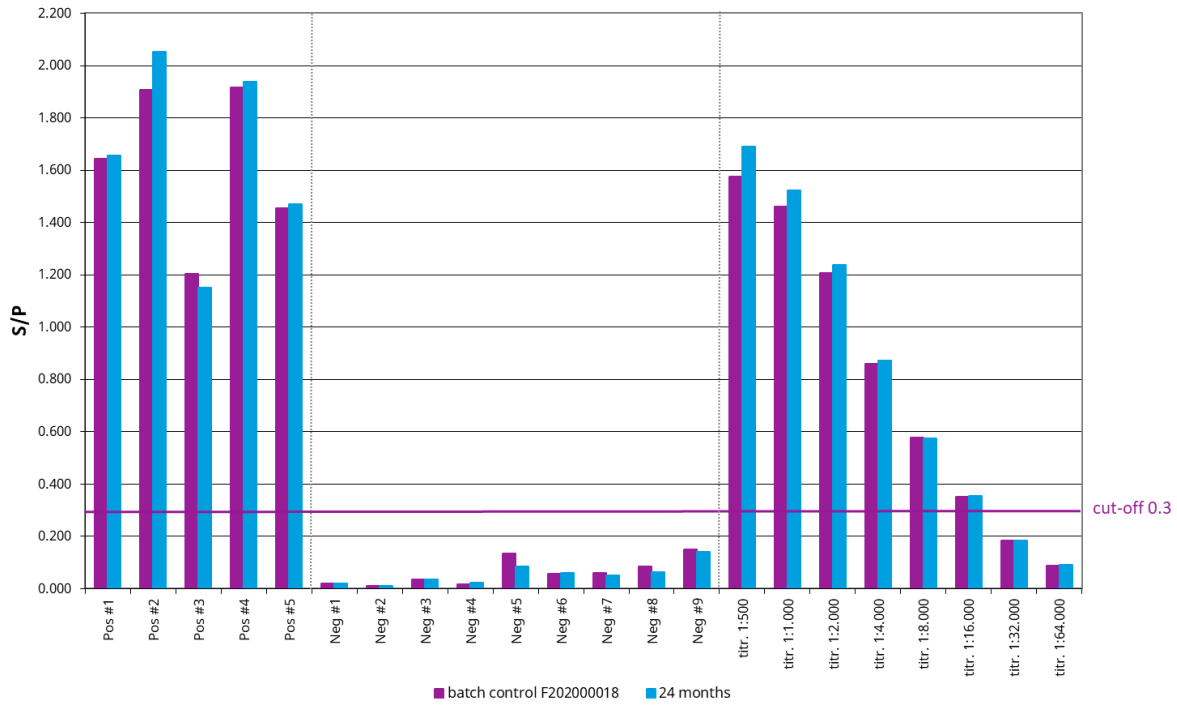

Figure 7. Real time stability data (S/P values, QC sample set) for the flocktype AIV Ab

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

A single protocol for all flocktype poultry assays from INDICAL

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Test components

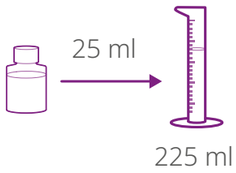
User-friendly, color-coded reagents

Sample Diluent	Ready-to-use	Positive Control	Ready-to-use
Conjugate	Ready-to-use	Negative Control	Ready-to-use
Substrate	Ready-to-use	Wash Buffer	Dilution 1:10
Stop Solution	Ready-to-use	ELISA plate	

Test preparation

flocktype ELISA preparation

Dilute Wash Buffer Concentrate



For one plate add 25 ml Wash Buffer concentrate to 225 ml of distilled water and mix

Sample Dilution

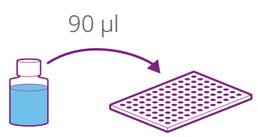
Step 1

Add 245 µl of Sample Diluent to predilution tube or predilution plate

Step 2

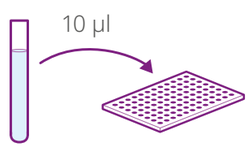
Add 5 µl of sample to predilution tube or predilution plate and mix

Step 3



Add 90 µl of Sample Diluent to ELISA plate

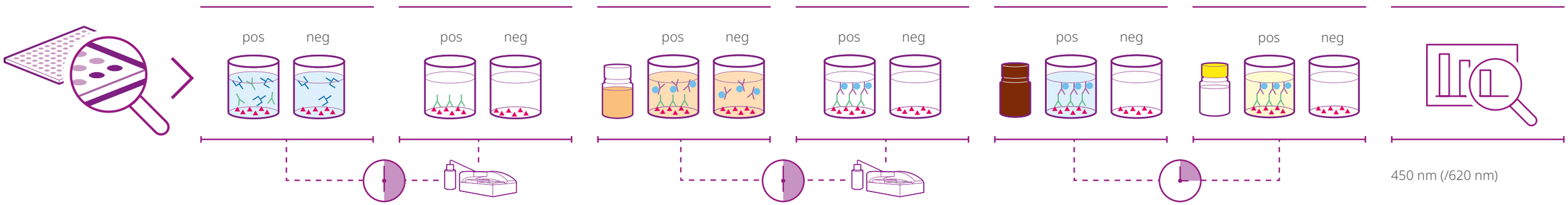
Step 4



Add 10 µl of prediluted sample to ELISA plate and mix

Test protocol

Harmonized and convenient indirect ELISA protocol



Incubation: 30 min 18-25°C (room temperature)

Incubation: 30 min 18-25°C (room temperature)

Incubation: 15 min 18-25°C (room temperature)

Test results

Easy result interpretation

		flocktype IBV Ab	flocktype IBDV Ab	flocktype NDV Ab	flocktype Salmonella Ab	flocktype Mycoplasma Ms Ab	flocktype AIV Ab
Cat. No.		FT274303 (5 plates)	FT274203 (5 plates)	FT275003 (5 plates)	FT275702 (2 plates)	FT274602 (2 plates)	FT274012 (2 plates)
Detects antibodies to		*Infectious Bronchitis Virus*	*Infectious Bursal Disease Virus*	*Newcastle Disease Virus*	*S.* Enteritidis & *S.* Typhimurium	*Mycoplasma synoviae*	Avian Influenza A Virus
Cut-off	Negative	S/P < 0.25	S/P < 0.2	S/P < 0.3	S/P < 0.2	S/P < 0.1	S/P < 0.3
	Suspect	-	-	-	S/P ≥ 0.2 and < 0.3	S/P ≥ 0.1 and < 0.2	-
	Positive	S/P ≥ 0.25	S/P ≥ 0.2	S/P ≥ 0.3	S/P ≥ 0.3	S/P ≥ 0.2	S/P ≥ 0.3
Validation criteria		Mean OD_{PC} – Mean OD_{NC} ≥ 0.2 Mean OD_{NC} ≤ 0.2			Mean OD_{PC} ≥ 0.7 Mean OD_{NC} ≤ 0.2		

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- Identification of vaccine breakthrough
- Quality control of vaccination to evaluate the level of coverage
- Improved and optimized vaccination program efficiency

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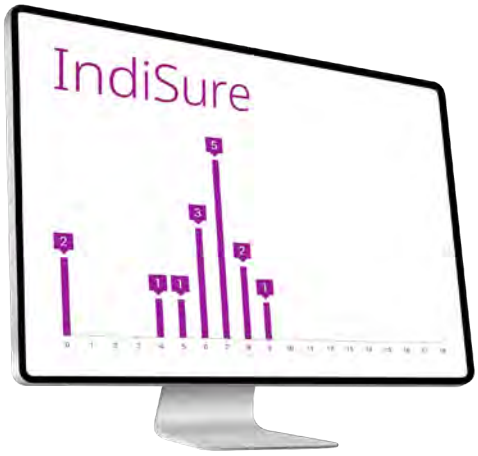
- Harmonized protocols with ready-to-use reagents and a flexible microplate system
- Interchangeable reagents for all flocktype assays*
- Suitable for low- to high-throughput application

*Sample Diluent, Wash Buffer, TMB Substrate and Stop Solution

flocktype poultry ELISAs †

Pathogen	***Infectious Bronchitis Virus***	***Infectious Bursal Disease Virus***	***Newcastle Disease Virus***	***S.* Enteritidis & *S.* Typhimurium**	***Mycoplasma synoviae***	**Avian Influenza A Virus**
Ready-to-use assay	flocktype IBV Ab	flocktype IBDV Ab	flocktype NDV Ab	flocktype Salmonella Ab	flocktype Mycoplasma Ms Ab	flocktype AIV Ab
Target	IBV-specific antibodies	IBDV-specific antibodies	NDV-specific antibodies	*S.* Enteritidis- & *S.* Typhimurium specific antibodies	Ms-specific antibodies	AIV-specific antibodies

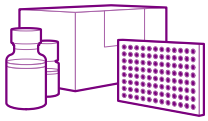
†Product availability/distribution: outside the U.S. and Canada



Powerful ELISA software

Our IndiSure ELISA Software allows you to easily interpret, store and navigate results. Multiple reports and tools help you visualize individual flock performance, predict trends and assess animal health.

- Versatile report structure for total visibility of flock health
- All reports, INDICAL ELISAs and key tools are preloaded
- Intuitive, LIMS-compatible workflow with easy report exporting



flocktype IBV Ab

The flocktype IBV Ab is a highly sensitive and specific ELISA for detection of antibodies to *Infectious Bronchitis Virus* (IBV) in serum and plasma samples from chicken. The assay reliably enables monitoring of humoral vaccination responses or IBV infections.

- 100% diagnostic specificity*
- 100% sensitivity in infected flock samples†
- 98.9% sensitivity in vaccinated flock samples‡
- User-friendly with ready-to-use, color-coded reagents
- Also detects D274 anti-serum
- Full results in 1.5 hours
- Excellent results in international ring trials§

**n* = 334 SPF sera.
† *n* = 80 infection samples from six infected flocks.
‡ *n* = 551 samples from 36 vaccinated flocks.
§ Data on file.

Excellent specificity and superior sensitivity in vaccination trials compared with other suppliers

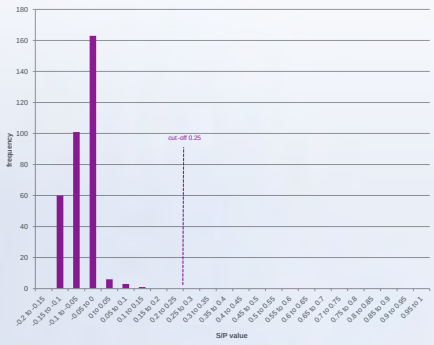


Figure 1: Frequency distribution for *n* = 334 chicken SPF sera tested using the flocktype IBV Ab.
Conclusion: The flocktype IBV Ab returned negative results for all samples tested, offering 100% diagnostic specificity.

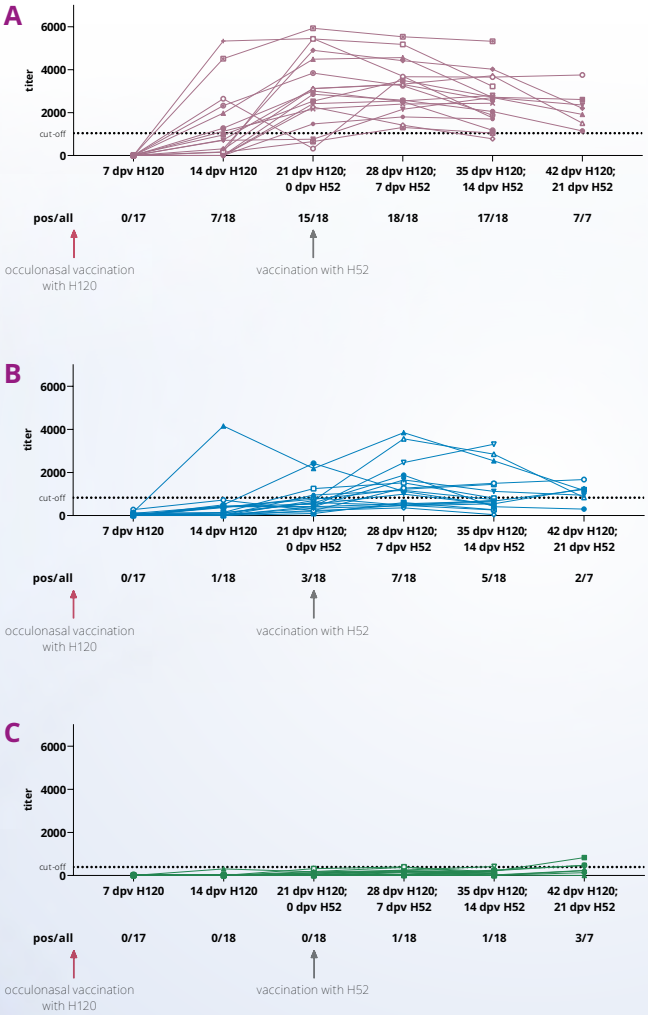


Figure 2: Temporal study of IBV-specific antibody content in serum from *n* = 18 vaccinated chicken. **A:** flocktype IBV Ab; **B:** Test from other supplier 1; **C:** Test from other supplier. The dashed line shows the cut-off for each test.
Conclusion: The flocktype IBV Ab provides sensitivity superior to assays from other suppliers.

Product availability/distribution: outside the U.S. and Canada

flocktype IBDV Ab

The flocktype IBDV Ab enables detection of antibodies to *Infectious Bursal Disease Virus* (IBDV) in serum and plasma samples from chicken. Infectious Bursal Disease (Gumboro disease) is characterized by immunosuppression – especially in young chicken – and is therefore of economic importance for the poultry industry. Vaccination of breeder flocks is an effective method of disease prevention. Detection of antibodies to IBDV using flocktype IBDV Ab enables monitoring of humoral vaccination responses or IBDV infections.

- 99.3% diagnostic specificity*
- 100% sensitivity in infected flock samples†
- 100% sensitivity in vaccinated flock samples‡
- Vaccination date prediction available
- User-friendly with ready-to-use, color-coded reagents
- Full results in 1.5 hours
- Excellent results in international ring trials§

**n* = 149 SPF sera.
† *n* = 75 infection samples from five infected flocks.
‡ *n* = 322 samples from 27 vaccinated flocks.
§ Data on file.

High diagnostic specificity and sensitivity in trials

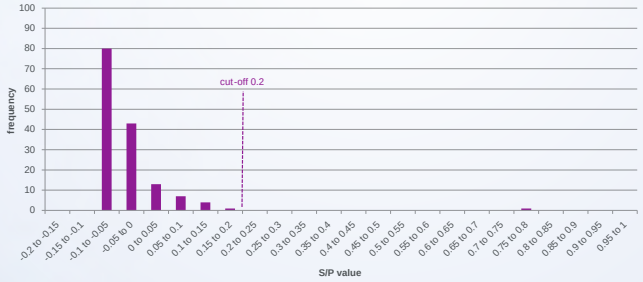


Figure 3: Frequency distribution for ***n*** = 149 chicken SPF sera tested using the flocktype IBDV Ab.
Conclusion: The flocktype IBDV Ab provides a 99.3% diagnostic specificity.

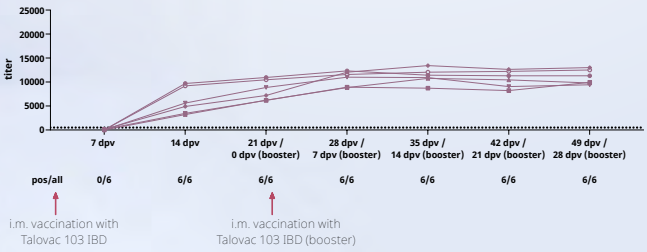


Figure 4: Temporal study of IBDV-specific antibody content in serum from ***n*** = 6 vaccinated chickens tested with the flocktype IBDV Ab.
Conclusion: This IBDV vaccination trial showed 100% sensitivity from 14 dpv.

Product availability/distribution: outside the U.S. and Canada

flocktype NDV Ab

The flocktype NDV Ab is a highly sensitive and specific solution for detection of antibodies to *Newcastle Disease Virus* (NDV) in serum and plasma samples from chicken. Newcastle Disease is an epizootic disease affecting many domestic and wild avian species. Prophylactic vaccinations are used to reduce the likelihood of outbreaks in domestic poultry populations. Detection of antibodies to NDV using the flocktype NDV Ab reliably enables monitoring of humoral vaccination responses or NDV infections.

- 100% diagnostic specificity*
- 100% sensitivity in infected flock samples†
- 98.7% sensitivity in vaccinated flock samples‡
- User-friendly with ready-to-use, color-coded reagents
- Full results in 1.5 hours
- Excellent results in international ring trials§

**n* = 108 SPF sera.
† *n* = 132 infection samples from 6 infected flocks
‡ *n* = 233 samples from 18 vaccinated flocks
§ Data on file.

Excellent diagnostic specificity and sensitivity

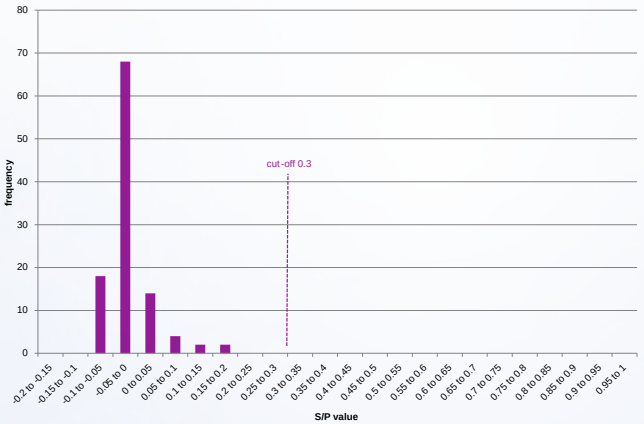


Figure 5: Frequency distribution for *n* = 108 chicken SPF sera tested using the flocktype NDV Ab. S/P <0.3 = negative; S/P ≥0.3 = positive. **Conclusion:** The flocktype NDV Ab provides a 100% diagnostic specificity.

Table 1: Results of *n* = 132 SPF sera derived from six infected chicken flocks tested using the flocktype NDV Ab.

				flocktype NDV Ab		
	Flock	**Age**	***n***	**pos**	**neg**	**Mean titer**
Infection samples	#1	unkn.	15	15	0	22694
	#2	unkn.	15	15	0	23706
	#3	unkn.	15	15	0	25829
	#4	unkn.	15	15	0	21946
	#5	42 d	60	60	0	23038
	#6	19 w	12	12	0	20795
Mean						23001
Sum			132	132	0	
Sensitivity					**100%**	

n = amount of samples, pos = positive, neg = negative, SD = standard deviation, CV = coefficient of variation (given in %), d = days, w = weeks, unkn. = unknown

Conclusion: The flocktype NDV Ab scored all tested infection samples correctly, offering 100% diagnostic sensitivity.

Product availability/distribution: outside the U.S. and Canada

flocktype Salmonella Ab

INDICAL's flocktype Salmonella Ab (BGVV-B 322) is a highly sensitive and specific assay that detects antibodies to *Salmonella* Enteritidis and *Salmonella* Typhimurium. Antibodies to the O-antigens 1, 4, 5, 9, and 12 are identified.

The flocktype Salmonella Ab is suitable for use with serum, plasma and egg yolk samples from chicken and turkey. Antibody diagnostics with the flocktype Salmonella Ab is the preferred screening method to detect *Salmonella* infections or humoral vaccination responses.*

- 98.9% diagnostic specificity†
- 96.4% sensitivity‡
- User-friendly with ready-to-use, color-coded reagents
- Full results in 1.5 hours

*Note: It is not possible to differentiate between antibodies present in samples due to immunization with a *Salmonella* vaccine or infection with *Salmonella* field strains.
† *n* = 90 SPF sera.
‡ *n* = 139 vaccinated broiler breeders.

flocktype Mycoplasma Ms Ab

The flocktype Mycoplasma Ms Ab is a highly sensitive and specific ELISA for the detection of antibodies to *Mycoplasma synoviae* (Ms) in serum and plasma samples from chicken and turkey.

Ms is found worldwide and causes chronic respiratory diseases in birds. Systemic infection leads to infectious synovitis. The severity of infection depends on secondary infections. *Mycoplasma* adheres to the respiratory epithelium and to erythrocytes. The cellular immune response is central to infections with Ms, although the production of specific antibodies may also be induced by infection and vaccination.

- 99.6% diagnostic specificity*
- 100% sensitivity†
- User-friendly with ready-to-use, colored reagents
- Full results in 1.5 hours

**n* = 272 SPF sera.
† *n* = 24 samples in Ms vaccination trial.

Product availability/distribution: outside the U.S. and Canada

flocktype AIV Ab

Developed for surveillance and vaccine compliance testing, the flocktype AIV Ab (FLI-B 435) is a screening ELISA for specific and sensitive detection of antibodies to *Avian Influenza A Virus* in serum and plasma samples from poultry, including chicken and turkey.

- 100% sensitivity*
- 96.8% specificity†
- User-friendly with ready-to-use, color-coded reagents
- Full results in 1.5 hours
- Part of INDICAL's broad range of Influenza A identification tools

**n* = 104 AIV antibody-positive samples.
† *n* = 432 AIV antibody-negative samples.

The flocktype AIV Ab shows high diagnostic specificity and delivers early antibody detection from day 6 after infection

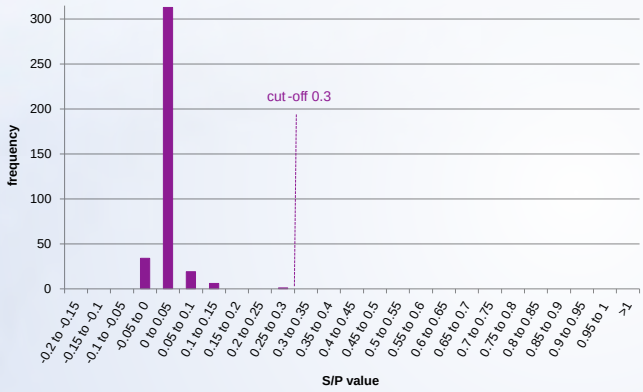


Figure 6: Distribution of n = 373 AIV antibody-negative field sera (pre-selected on their negative test result in an ELISA from another supplier)
Conclusion: 100% specificity for AIV-antibody negative field sera.

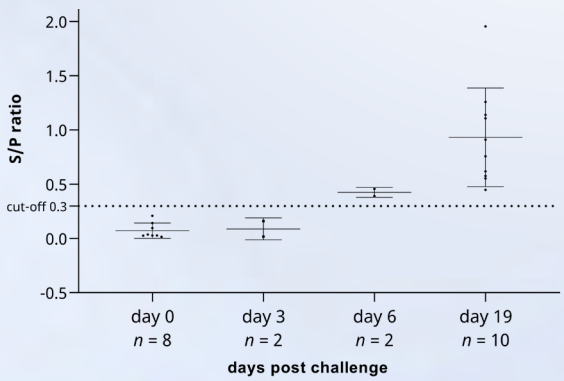


Figure 7: Course of seroconversion after vaccination and subsequent challenge with an AIV H7N1 strain.
Conclusion: Antibodies to AIV H7N1 are detected from day 6 post challenge. Therefore, the flocktype AIV Ab is a reliable tool for early detection of AIV-specific antibodies.

Product availability/distribution: outside the U.S. and Canada

Peace of mind for your flocks' health

With over two decades of experience, INDICAL is a leading provider of diagnostic workflows, associated protocols, and expert technical support. We are committed to protecting animal and human health by enabling you to reliably identify and monitor pathogens that affect the health of your flocks.

Accessible advice and support: Our team of experienced veterinarians, biologists, and application specialists stand ready to answer your questions.

Ordering information

Pathogen investigated	Product*	Technology	Number of reactions	Cat. No.
Infectious Bronchitis Virus (IBV)	flocktype IBV Ab	Indirect ELISA	480 tests (5 ELISA plates)	FT274303
Infectious Bursal Disease Virus (IBDV)	flocktype IBDV Ab	Indirect ELISA	480 tests (5 ELISA plates)	FT274203
Newcastle Disease Virus (NDV)	flocktype NDV Ab	Indirect ELISA	480 tests (5 ELISA plates)	FT275003
Salmonella Enteritidis and *Salmonella* Typhimurium	flocktype Salmonella Ab	Indirect ELISA	192 tests (2 ELISA plates)	FT275702
Mycoplasma synoviae (Ms)	flocktype Mycoplasma Ms Ab	Indirect ELISA	192 tests (2 ELISA plates)	FT274602
Avian Influenza A Virus	flocktype AIV Ab	Indirect ELISA	192 tests (2 ELISA plates)	FT274012

*Product availability/distribution: outside the U.S. and Canada

Product	Short description	Cat. No.
IndiSure ELISA Software Demo licence	For e.g., calculation and management of results using ELISAs from INDICAL	SW270001
IndiSure ELISA Software Single-user license for one year	For e.g., calculation and management of results using ELISAs from INDICAL	SW270002

Get in touch with our experts: **www.indical.com**

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Connect with us on LinkedIn: **www.linkedin.com/company/indical**

INDICAL BIOSCIENCE

INDICAL BIOSCIENCE GmbH
Deutscher Platz 5b
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July 2022 | EN

virotype® BTV pan/8 2.0 RT-PCR Kit

Handbook

For detection of RNA from *Bluetongue Virus* (BTV) and European serotype BTV-8

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 114

REF 100 reactions (Cat. no. VT280465)

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

INDICAL BIOSCIENCE

Contents

Kit contents

virotype BTV pan/8 2.0 RT-PCR Kit **Cat. no.** **Number of reactions**	**(100)** **VT280465** **100**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1 x 800 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
Handbook	1

Intended use

The virotype BTV pan/8 2.0 RT-PCR Kit is intended for the detection of *Bluetongue Virus* (BTV) RNA and differentiation of European serotype BTV-8 specifically, in ruminant whole blood and blood pools (preferred with anticoagulants, for example EDTA-blood) and tissue samples (spleen, lymph nodes) from cattle, sheep and goats.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 114) 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
	Protect from light
	For samples from cattle, sheep and goats

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype BTV pan/8 2.0 RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype BTV pan/8 2.0 RT-PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (> 3x), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

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**.

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

Introduction

Bluetongue is an infectious, non-contagious disease of ruminants. The agent is the *Bluetongue Virus* (BTV), a double-stranded RNA virus of the genus *Orbivirus* of the family *Reoviridae* which includes 36 known serotypes including atypical BTV. BTV is widely distributed around the world. Sheep, cattle and goats are mainly affected by the disease. Clear clinical signs are usually seen only in sheep. In severe cases the tongue may show intense hyperemia and becomes cyanotic (Bluetongue).

BTV serotype 8 (BTV-8) is of epidemiological importance in Central Europe and cause of recent major Bluetongue Disease outbreaks. The virus is transmitted by certain midges of the genus *Culicoides*. Furthermore, the virus can be spread by contaminated needles and surgery equipment.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is identified using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward.

The virotype BTV pan/8 2.0 RT-PCR Kit contains all of the necessary reagents for the detection of BTV RNA, including a Positive and Negative Control. With this kit, both, reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The virotype BTV pan/8 2.0 RT-PCR Kit uses three specific primer/probe combinations:

- FAM™ fluorescence for RNA of all known BTV serotypes (pan BTV)
- Cy®5 fluorescence for RNA of European serotype 8 (BTV-8)
- JOE™ fluorescence for the endogenous Internal Control (EC; β-actin present within the sample)

A Positive Control contains BTV-8 RNA and allows the control of the denaturation step since the successful denaturation of the viral double-stranded RNA is a prerequisite for amplification.

RNA extraction

The virotype BTV pan/8 2.0 RT-PCR Kit can be used for the detection of BTV RNA from ruminant whole blood (preferred with anticoagulants, e.g., EDTA-blood) and tissue samples (spleen, lymph nodes) from cattle, sheep and goats.

Due to the high sensitivity of the test, pools of up to 10 individual blood samples may be analyzed. However, the optimum pool size depends on the regional prevalence for BTV.

Note: For use in Germany the specifications described in the „*Amtliche Methodensammlung*“ apply.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material.

INDICAL offers a range of validated kits for the extraction of RNA from animal samples.

Extraction based on magnetic beads:

- **IndiMag® Pathogen Kit** (SP947457)
- **IndiMag Pathogen Kitw/o plastics** (SP947257)
- **IndiMag Pathogen IM48 Cartridge** (SP947654P608, SP947654P224)
- **IndiMag Pathogen KF96 Cartridge** (SP947855P196)

Extraction based on spin columns:

- **IndiSpin® Pathogen Kit** (SP54104, SP54106)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161)

If real-time RT-PCR is not performed immediately after extraction, store the RNA at -20°C or at -70°C for longer storage.

For further information on automated and manual extraction of BTV RNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

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.

- Pipets
- Nuclease-free, aerosol-resistant pipet tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- Nuclease-free (RNase/ DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen real-time cycler

Important notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipet tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting as assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted viral RNA. A positive control serves to prove the functionality of the pathogen assay, e.g., the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with

the virotype BTV pan/8 2.0 RT-PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, one extraction and amplification control assay is included in the test kit.

An endogenous internal control (EC) detects the β-actin gene present within the sample. This allows extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for detection of RNA from *Bluetongue Virus* and BTV-8

Important points before starting

- Please read „Important notes“ on page 10 before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- RNA is unstable. Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

1. Pipet 5 µl of RNA samples, Positive Control, and Negative Control into individual reaction tubes. Cover the reaction tubes (e.g., with PCR sealing foil).

   Include positive and negative control reactions.

   Positive Control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.

   Negative Control: Use 5 µl of the negative control (Negative Control) instead of sample RNA.

2. Denature the samples and Controls for 5 min at 98°C in a 96-well plate standard cycler with a heated lid.
3. Immediately cool down on ice water or liquid nitrogen for at least 20 s. Then store on ice or in a cooling device.
4. Before use, mix the Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
5. Pipet 8 µl of the Master Mix into each reaction tube. Thus, the final volume of a test is 13 µl (Table 1).

Table 1. Preparation of reaction mix

Component	**Volume**
Master Mix	8 µl
Sample	5 µl
Total volume	**13 µl**

6. Close the reaction tubes with the corresponding caps.
7. Invert the closed tubes several times until mixed thoroughly and spin down briefly.
8. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ internal control	Reporter
pan BTV	FAM
BTV-8	Cy5
Endogenous internal control (EC)	HEX/ JOE™[1]
Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.
2 Internal reference for use with ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

9. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time RT-PCR protocol for BTV pan/8 2.0

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/ Extension*	60°C	30 s	

* Fluorescence data collection, approximate run time 67 min (CFX96, Bio-Rad™)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM and HEX/ JOE channels with a C_T[1] < 35. If no FAM and no Cy5 signals of the Positive Controls are measured the denaturation and cooling steps were insufficient and the testing should be repeated. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 17.

The sample is positive for pan BTV and BTV-8, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM, Cy5 and HEX/JOE channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal in any of the channels.

Note that very high concentrations of BTV-8 RNA or presence of inhibitors in the sample may lead to a reduced HEX/JOE signal or no HEX/JOE signal due to competition with the internal control.

[1] Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

The sample is positive for pan BTV and negative for BTV-8, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM and HEX/JOE channel, but not in the Cy5 channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal in any of the channels.

Note that very high concentrations of BTV-8 RNA or presence of inhibitors in the sample may lead to a reduced HEX/JOE signal or no HEX/JOE signal due to competition with the internal control.

The sample is negative for both, pan BTV and BTV-8, and the assay is valid, if the following criteria are met:

- The sample yields a signal only in the HEX/JOE channel.
- The Positive Control yields a signal in all channels.
- The Negative Control yields no signal in any of the channels.

A positive HEX/JOE signal rules out the possibility of PCR inhibition and/ or incorrect RNA extraction as the internal control is amplified.

The sample results are inconclusive, and the assay is invalid, if the following criteria are met:

- The sample yields no signal in any of the fluorescence channels.

If no signal is detected in the FAM (pan BTV), Cy5 (BTV-8) and the HEX/JOE (endogenous Internal Control, EC) channels, the result is inconclusive. The absence of a signal for the housekeeping gene indicates strong PCR inhibition and/or other malfunctions, e.g., during extraction.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease-free water, to repeat the RNA extraction procedure, or repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in all the channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect RNA denaturation or incorrect cycling conditions.

Table 4. Results interpretation table*

Sample result	FAM (pan BTV)	Cy5 (BTV-8)	HEX (EC)
pan BTV positive	X		(X)
pan BTV and BTV-8 positive	X	X	(X)
BTV negative			X
Inconclusive			

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the FAM, Cy5 and HEX/JOE channels. The Negative Control must yield no signal in any channel. For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 15.

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.

Notes

Notes

Limited License Agreement for virotype BTV pan/8 2.0 RT-PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. INDICAL grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Change index

Handbook	Version	Change
HB-2578-EN-001	July 2022	Product launch

INDICAL BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**

January 2020 | EN

IndiSpin® Pathogen Kit Handbook

For the purification of viral RNA and DNA and bacterial DNA from animal whole blood, serum, plasma, other body fluids, swabs and washes, and tissue

REF IndiSpin Pathogen Kit (cat no SP54104),
formerly QIAamp® cador® Pathogen Mini Kit (50)

REF IndiSpin Pathogen Kit (cat no SP54106),
formerly QIAamp cador Pathogen Mini Kit (250)

Manufactured by QIAGEN® GmbH for INDICAL BIOSCIENCE
INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

IndiSpin Pathogen Kit	**(50)**	**(250)**
Cat. no.	**SP54104**	**SP54106**
Number of preps	**50**	**250**
IndiSpin Columns	50	250
Buffer VXL[1]	1 x 6 ml	1 x 30 ml
Buffer ACB (concentrate)[1,2]	1 x 12 ml	1 x 60 ml
Proteinase K	1 x 1.25 ml	1 x 6 ml
Carrier RNA (poly A)	1 x 310 µg	1 x 310 µg
Buffer AW1 (concentrate)[1,3]	1 x 19 ml	1 x 98 ml
Buffer AW2 (concentrate)[3]	1 x 17 ml	1 x 81 ml
Buffer AVE	1 x 20 ml	2 x 20 ml
Quick-Start Protocol (PCard)	1	1

1 CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.
2 Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.
3 Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Storage

IndiSpin columns and buffers can be stored dry at room temperature (15-25ºC) until the expiration date stated on the kit box without affecting performance.

Lyophilized Carrier RNA can be stored at room temperature until the expiration date stated on the kit box. For use, lyophilized Carrier RNA should be dissolved in Buffer AVE and then added to Buffer VXL, as described in “Preparing reagents”, on page 17. This Carrier RNA/Buffer AVE/Buffer VXL mix solution should be prepared fresh and is stable at room temperature for up to 48 hours. Unused Carrier RNA dissolved in Buffer AVE should be immediately frozen in aliquots at -30 to -15°C. Do not subject aliquots of Carrier RNA to more than 3 freeze-thaw cycles.

Proteinase K can be stored at room temperature (15-25°C). To store for extended periods of time, or if the ambient temperature often exceeds 25°C, we recommend storing at 2-8°C.

Intended use

The IndiSpin Pathogen Kit is intended for the extraction of pathogen nucleic acids (viral RNA and DNA, and bacterial DNA) from animal whole blood, serum, plasma, other body fluids, swabs, washes, and tissue (homogenate).

For molecular biology applications.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

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: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffer VXL and Buffer AW1 contain guanidine hydrochloride, and Buffer ACB contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality control

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

Introduction

The IndiSpin Pathogen Kit enables the efficient purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types, including whole blood, serum, plasma, swabs, washes, and tissue (see “Starting material” on page 12). The extracted nucleic acids are free of proteins, nucleases, and other impurities, and are ready for use in downstream applications, such as real-time PCR-based pathogen identification.
However, specific combinations of sample types and pathogens should be validated by the user.

The kit is not intended for host RNA or host DNA preparation.

Principle and procedure

Samples are lysed under highly denaturing conditions at room temperature (15 - 25°C) in the presence of Proteinase K and Buffer VXL, which together ensure the inactivation of nucleases. Adding Buffer ACB adjusts the binding conditions for the copurification of DNA and RNA. The lysate is then transferred to an IndiSpin Column. During centrifugation, nucleic acids are adsorbed onto the silica membranes while contaminants pass through. Two efficient wash steps remove the remaining contaminants and enzyme inhibitors, and nucleic acids are eluted in Buffer AVE.

Performance is not guaranteed for every combination of starting material and pathogen species and must be validated by the user. Some samples may require a pretreatment (see Table 1, page 9).

Nucleic acid purification protocol

The “Purification of pathogen nucleic acids from fluid samples” protocol (page 21) is optimized for purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from up to 200 µl of fluid material. Suitable starting materials for **direct processing** using this method include:

- whole blood
- serum
- plasma
- oral fluid
- body cavity fluids (e.g., peritoneal, synovial, cerebrospinal)
- liquid extracts from swabs (e.g., nasal, pharyngeal, and cloacal* swabs)
- wash fluids (e.g., from bronchoalveolar lavages)
- other fluids, such as urine or feces suspensions*

Most sample types can be directly processed without pretreatment. However, depending on the starting material and the target pathogen, one of the pretreatment protocols may be needed. For samples that require a pretreatment prior to nucleic acid purification, Table 1 on page 9 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

The lysis and binding solutions used in the procedure are Buffer VXL and Buffer ACB. Please pay attention to the information given under “Safety information”, page 5.

* The processing of samples with a high inhibitor content, such as urine and feces, may require a reduction in sample input volume or further measurements. For further pretreatment recommendations, contact INDICAL support (**support@indical.com**).

Pretreatments

The pretreatments mentioned in this handbook are optimized for specific combinations of starting material and target pathogens. The choice of pretreatment depends on the workflow focus, and is to be followed by nucleic acid purification.

Table 1 on page 9 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components, which are indicated in each pretreatment protocol.

Table 1: Overview of available pretreatment protocols

Sample	Target	Pretreatment	handbook
Fluids (e.g., whole blood, serum, plasma, swab or wash fluid, pretreated tissue)	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	-	-
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria[1]	**Pretreatment B1** for difficult-to-lyse bacteria in whole blood or pretreated tissue	**HB-2533**
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria[1]	**Pretreatment B2** for difficult-to-lyse bacteria in body fluids[2]	**HB-2534**
High volume cell-free fluids (for increased sensitivity)	DNA of easy-to-lyse bacteria[1]	**Pretreatment B3** for difficult-to-lyse bacteria in body fluids[2]	**HB-2549**

Tissue (e.g., liver, spleen, kidney, lymph node)	Pathogen nucleic acids	**Pretreatment T1** Mechanical disruption of tissue	**HB-2535**
	Viral DNA[3], bacterial DNA[4]	**Pretreatment T2** Enzymatic digestion of tissue	**HB-2536**
Rapid Partial Disruption of tissue	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	**Pretreatment T3**	**HB-2537**
Tissue containing high amount of lipids and/or nucleases (e.g. brain, pancreas)	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	**Pretreatment T4**	**HB-2538**
Feces	Viral RNA and DNA	**Pretreatment F1** Non-lysing suspension method	**HB-2513**
	Bacterial DNA[1] and viral DNA	**Pretreatment F2** Lysing suspension method	**HB-2514**
	Mycobacterium avium subsp. *paratuberculosis* (MAP) DNA	**Pretreatment F-MAP**	**HB-2503**
Filter paper cards		**Pretreatment C1**	**HB-2520**
Swabs (tracheal, oropharyngeal, blood)		**Pretreatment S1**	**HB-2516**

1 Gram-positive bacteria are difficult to lyse due to their rigid cell wall. Many Gram-negative bacteria are easy to lyse, but some are not and will also benefit from Pretreatment B1 or B2.
2 Not suitable for whole blood.
3 Not suitable for viral RNA as the lysis conditions do not sufficiently conserve RNA integrity.
4 For difficult-to-lyse bacteria, subsequently use Pretreatment B1.

For further information on Pretreatments website www.indical.com/handbooks contact INDICAL Support at **support@indical.com**.

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.

- Pipettors and disposable pipette tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipette tips with aerosol barriers
- Multidispenser
- Ethanol (96-100%)*
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Vortexer

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important notes

Starting material

Do not overload the IndiSpin Column, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. For samples with very high host nucleic acid contents (e.g., for certain tissues, such as spleen or blood samples with highly increased cell counts), use less than the maximum amount of sample recommended in the protocol or pretreatments. In some downstream applications such as PCR and RT-PCR, very high background concentrations of nucleic acids may impair the reaction. Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

Avoid transferring material to the IndiSpin Column that could subsequently clog the membrane (e.g., blood clots, solid tissue, swab fibers).

Highly viscous fluids may require treatment to reduce their viscosity, to allow for efficient extraction of pathogen nucleic acids. Please contact INDICAL support at **support@indical.com** for recommendations.

Avoid repeated thawing and freezing of samples, since this may reduce nucleic acid yield and quality.

Animal whole blood

Blood samples treated with EDTA, citrate, or heparin as anticoagulant can be used for nucleic acid purification. Samples can be either fresh or frozen, provided that they have not been freeze-thawed more than once. Freeze-thawing more than once can lead to denaturation and precipitation of proteins, resulting in potential reduction in viral titers, and therefore, reduced yields of viral nucleic acids.

After collection and centrifugation, whole blood samples can be stored at 2-8ºC for up to 6 hours. For longer storage, we recommend freezing aliquots at -30 to -15ºC or at -70ºC.

We recommend using 50-200 µl blood containing non-nucleated erythrocytes. However, highly elevated cell counts due to inflammatory or neoplastic diseases may strongly increase the host nucleic acid content of a sample. In this case, reduction of sample input to 50 µl may improve results in downstream assays, particularly in RT-PCR. If using less than 200 µl blood, adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 50 µl blood and adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

Animal serum, plasma, other body fluids, swab, and wash specimens

Frozen plasma or serum must not be thawed more than once before processing.

We recommend storing swabs in transport media; for example, viral transport media (VTM) or brain-heart infusion broth (BHI). Remove the swab and squeeze out the liquid by pressing the swab against the inside of the storage tube. For extraction of viral RNA or DNA, we recommend centrifuging the swab media briefly to ensure any residual solid materials are removed.

Note: Solid pieces remaining in the sample fluid may aggregate on the IndiSpin Column, which may decrease nucleic acid yield.

Up to 200 µl serum, plasma, other body fluid, swab media supernatant, or wash fluid can be processed.

Carrier RNA must be used in the nucleic acid purification protocol to prevent the loss of nucleic acids during the procedure (see page 15 for information on the use of Carrier RNA).

The processing of samples with very high inhibitor contents, such as urine or fecal suspensions, may require a reduction in sample input volume and/ or an extra pretreatment to remove inhibitors. To reduce the input volume, use 25-50 µl of the sample and adjust the volume to 200 µl with PBS or 0.9% NaCl.

For extraction of bacterial DNA, the input volume can be increased to more than 200 µl, e.g., 1.5 ml for increased sensitivity of bacterial detection. Gram-negative bacteria in cell-free fluids can be concentrated by centrifugation of higher volumes. Resuspend pellets in PBS and use 200 µl as starting volume. See Pretreatment B2 for extraction of DNA from difficult-to-lyse bacteria and Pretreatment B3 for extraction of DNA from easy-to-lyse bacteria.

Animal tissues

When working with tissue samples, mechanical or enzymatic disruption of the tissue structure is the prerequisite for liberation of cells, subsequent release of nucleic acids, and membrane permeability of the material.

Different tissue types can vary widely with regard to texture and rigidity, cell types, and content of host nucleic acids and inhibitory substances. In addition, the localization of pathogen nucleic acids in the tissue may vary depending on tissue type, pathogen, and stage of infection. Therefore, suitability of the pretreatment protocols in this handbook should be evaluated for each new combination of tissue and pathogen. Additional pretreatments for tissue samples are available at INDICAL Support, including a rapid protocol and recommendations for difficult tissues.

Up to 25 mg of fresh or frozen tissue can be used as a starting amount. For tissues with a very high number of cells for a given mass of tissue, such as spleen, a reduced amount of starting material (5-10 mg) should be used.

Yields of nucleic acids

For samples containing a low amount of cells (e.g., serum and plasma), the yield of viral nucleic acids obtained can be below 1 µg and is therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with Carrier RNA may contain much more Carrier RNA than target nucleic acids. The IndiSpin Pathogen Kit recovers total nucleic acids. Therefore, cellular DNA and RNA will be co-purified from any cells in the sample along with viral RNA and DNA, and bacterial DNA, and cannot be distinguished using spectrophotometric measurements. We recommend using quantitative amplification methods such as quantitative real-time PCR or real-time RT-PCR to determine pathogen nucleic acid yields.

Using Carrier RNA and internal controls

Carrier RNA

We recommend adding Carrier RNA to fluids containing low amounts of cells such as serum, plasma, swab media, and wash fluid.
This enhances adsorption of viral RNA and DNA to the silica membranes, which is especially important when the target molecules are not abundant. In addition, an excess of Carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergents in the lysis buffer. Not using Carrier RNA may decrease the recovery of viral nucleic acids. Do not add Carrier RNA to whole blood and tissue samples, or other

samples containing a high amount of cells.

Internal Control

Use of an internal control, such as the intype IC-DNA or intype IC-RNA is optional, depending on the amplification system of choice. If the IndiSpin Pathogen Kit is used in combination with amplification systems that employ an internal control, introduction of these internal controls may be required during the purification procedure, to monitor the efficiency of sample preparation and downstream assay.

Add unprotected internal control nucleic acids (e.g., plasmid DNA or in vitro transcribed RNA) to VXL mixture only. Do not add these internal control nucleic acids directly to the sample.

The amount of internal control added depends on the assay system and the elution volume. Evaluation of the correct amount of internal control nucleic acid must be performed by the user. Refer to the manufacturer's instructions to determine the optimal concentration of internal control or contact INDICAL Support (**support@indical.com**) for further information.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at 2-8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at -30 to -15°C, or even at -70°C in the case of RNA.

Handling RNA

RNases are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate

and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents

Carrier RNA stock solution

For use, lyophilized Carrier RNA should first be dissolved in Buffer AVE. Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized Carrier RNA to obtain a stock solution of 1 µg/µl. Add this solution to Buffer VXL mixture as in Table 5 on page 22. Unused Carrier RNA dissolved in Buffer AVE should be frozen in aliquots at -30 to -15°C. Aliquots of Carrier RNA should not be subjected to more than 3 freeze-thaw cycles.

Adding Carrier RNA to Buffer VXL

We recommend adding Carrier RNA to fluids containing a low amount of cells such as serum, plasma, swabs media, and wash fluid. Do not add Carrier RNA to samples with a high cell content such as whole blood and tissue because high amounts of background nucleic acids may negatively influence downstream applications such as RT-PCR.

Carrier RNA dissolved in Buffer AVE is added to Buffer VXL so that 1 µg carrier RNA is present in each sample.

Note: 100 µl of Buffer VXL containing dissolved Carrier RNA is used per preparation.

Important note: Carrier RNA does not dissolve in Buffer VXL. It must first be dissolved in Buffer AVE.

The Buffer VXL solution containing dissolved Carrier RNA should be prepared fresh and is stable at room temperature (15-25ºC) for up to 48 hours.

Proteinase K

The IndiSpin Pathogen Kit contains ready-to-use Proteinase K supplied in a specially formulated storage buffer. The activity of the Proteinase K solution is 600 mAU/ml.

Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15-25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, we recommend storing Proteinase K at 2-8°C.

Buffer ACB

Buffer ACB is supplied as a concentrate. Before using for the first time, add Isopropanol (100%) as indicated on the bottle and in Table 2, page 18. Tick the check box on the bottle label to indicate that Isopropanol has been added. Mix well after adding Isopropanol.

Table 2: Preparation of Buffer ACB

No of preps	ACB concentrate	Isopropanol	Final volume
50	12 ml	8 ml	20 ml
250	60 ml	40 ml	100 ml

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add Ethanol (96-100%) as indicated on the bottle and in Table 3, page 19. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature
(15-25°C) for up to 1 year. Mix well after adding Ethanol.

Table 3: Preparation of Buffer AW1

No of preps	ACB concentrate	Ethanol	Final volume
50	19 ml	25 ml	44 ml
250	98 ml	130 ml	228 ml

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time add Ethanol (96-100%) as indicated on the bottle and in Table 4, page 19. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding Ethanol.

Table 4: Preparation of Buffer AW2

No of preps	ACB concentrate	Ethanol	Final volume
50	17 ml	40 ml	57 ml
250	81 ml	190 ml	271 ml

Handling Buffer AVE

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by

inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

Protocol: Purification of pathogen nucleic acids from fluid samples

This protocol is for the purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from fluid samples or pretreated tissue samples. The sample volume can be up to 200 µl.

Important points before starting

- Before beginning the procedure, read “Important notes” (page 12).
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions in “Preparing reagents” (page 17).
- Check that Buffer VXL or Buffer ACB does not contain a white precipitate. If necessary, incubate Buffer VXL or Buffer ACB for 30 minutes at 37°C with occasional shaking to dissolve precipitate.

Things to do before starting

- If necessary, thaw and equilibrate samples at room temperature (15-25°C).
- If the volume of the sample is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- If necessary, prepare a mixture of Buffer VXL and Carrier RNA according to Table 5 on page 22, for use in step 3 of the procedure.

  Important: Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates. Follow the procedure as described below (pipetting Proteinase K into the wells, followed by sample and then Buffer VXL/Carrier RNA mixture).

Note: Prepare a volume of the Buffer VXL/Carrier RNA/Internal Control mixture that is 10% greater than that required for the total number of sample purifications to be performed.

Table 5: Buffer VXL mixture preparation

Reagent	Number of samples *		
	1	12	30
Buffer VXL	100 µl	1.32 ml	3.3 ml
Carrier RNA (1 µg/µl)	1 µl	13 µl	33 µl

* The volume prepared is 110% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

Procedure

1. **Pipet 20 µl Proteinase K into a 2 ml microcentrifuge tube (not provided).**
2. **Add 200 µl fluid sample to the Proteinase K.**

   Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS or 0.9% NaCl.
3. **Add 100 µl Buffer VXL. Close the cap and mix by pulse-vortexing.**

   To ensure sufficient lysis, thoroughly mix the sample and Buffer VXL to yield a homogenous solution. If using sample fluid containing Buffer ATL, e.g., after enzymatic digestion of tissue, precipitates may form. Precipitates can be dissolved by brief incubation at 56°C. However, they have no influence on subsequent protocol steps.

   Note: If processing cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl of Buffer VXL before use. Do not add Carrier RNA if processing cell-rich samples, such as whole blood and tissue.
4. **Incubate at 20-25°C for 15 min.**
5. **Briefly centrifuge the 2 ml tube to remove drops from inside the lid.**
6. **Add 350 µl Buffer ACB to the sample, close the cap, and mix thoroughly by pulse-vortexing.**

   Ensure that Isopropanol was added to the Buffer ACB concentrate before first use.
7. **Briefly centrifuge the 2 ml tube to remove drops from inside the lid.**
8. **Transfer the lysate from step 7 to the IndiSpin Column placed in a 2 ml collection tube without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the IndiSpin**

Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (up to 20,000 x *g*; 14,000 rpm) until the IndiSpin Column is empty.

9. **Open the IndiSpin Column, and add 600 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the IndiSpin Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

10. **Open the IndiSpin Column, and add 600 µl Buffer AW2 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the IndiSpin Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

11. **Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min to dry the membrane.**

12. **Place the IndiSpin Column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Open the IndiSpin Column, and add 50-150 µl Buffer AVE to the center of the membrane. Close the cap, and incubate at room temperature (15-25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**

    Important: Ensure that the elution buffer is equilibrated at room temperature. If elution is performed with a small volume (<75 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA. Elution volume is flexible and can be adapted according to requirements of the downstream application.

    To reduce noise, the centrifugation speed for elution can be set to 6000 x *g*. If this is done, the recovered eluate volume will be

approximately 5 µl less than elution buffer volume applied onto the column.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise.

For more information or help please contact INDICAL Support at **support@indical.com**.

		Comments and suggestions
Little or no pathogen DNA or RNA in the eluate		
1	Buffer ACB prepared incorrectly	Check that Buffer ACB concentrate was diluted with the correct volume of Isopropanol, as indicated on the bottle. Use 100% Isopropanol. Repeat the purification protocol with new samples.
2	Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW or Buffer AW2 concentrate was diluted with the correct volume of 96–100% ethanol, as indicated on the bottle. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol with new samples.
3	Insufficient sample lysis	Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh Proteinase K (see storage recommendations on page 4). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. In this case, mix the samples thoroughly after addition of Proteinase K and incubate for 15 min at 70°C.
4	IndiSpin Column not incubated with Buffer AVE before elution	After addition of Buffer AVE, the IndiSpin Column should be incubated at room temperature (15-25°C) for 1 min.
5	Carrier RNA not added to Buffer VXL	For samples containing a low number of cells, not using Carrier RNA may decrease the recovery of pathogen nucleic acids. For these samples, reconstitute Carrier RNA in Buffer AVE and add reconstituted Carrier RNA too Buffer VXL as

		described on page 22. Repeat the purification procedure with new samples.
6	Degraded Carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at -30 to -15°C or underwent multiple freeze-thaw cycles. Alternatively, Buffer VXL/Carrier RNS mixture was stored for more than 48 h at 2-8°C. Prepare a new tube of Carrier RNA dissolved in Buffer AVE. Repeat the purification procedure with new samples.
7	Buffer VXL/Carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipette.
8	RNase contamination in Buffer AVE	If tubes containing Buffer AVE are accessed repeatedly, be careful to not introduce RNases which can degrade viral RNA. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples.
9	Nucleic acids in samples already degraded prior to purification	Samples were freeze-thawed more than once or stored at room temperature (15-25ºC) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
DNA or RNA does not perform well in downstream applications		
1	Little or no DNA or RNA in the eluate	See “Little or no pathogen DNA or RNA in the eluate” (above) for possible reasons.
2	Excessive eluate in the amplification reaction	Some sample types may contain high amounts of background nucleic acids (e.g., animal whole blood, tissue) or PCR inhibiting substances (feces). High amounts of background nucleic acids may inhibit amplification reactions, and removal of inhibitors might not be complete without special treatment. Reduce the amount of sample input or/and the amount of eluate added to the amplification reaction.
3	Excessive Carrier RNA in the eluate	Determine the maximum amount of Carrier RNA suitable for your amplification reaction. Adjust the

		concentration of Carrier RNA solution added to the Buffer VXL accordingly.
4	Performance of purified nucleic acids in assays varies with aging of reconstituted wash buffers	Salt and ethanol components of Buffer AW1 or Buffer AW2 may have separated out after being left for a ling period between preparation. Always mix buffers thoroughly before each preparation.
5	Residual ethanol in the eluate	Use the drying step (step 11) in the protocol: “Purification of pathogen nucleic acids from fluid samples” (page 24).
Precipitate in buffers		
1	Precipitate in Buffer VXL or Buffer ACB	Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 30 min at 37°C, with occasional shaking.
2	Precipitate in sample-Buffer VXL mixture	If using sample fluid containing Buffer ATL, e.g., after enzymatic digestion of tissue, precipitate may form after addition of Buffer VXL to the sample. The precipitate does not influence subsequent protocol steps and can be dissolved by brief incubation at 56°C.

Order information

Product name	Cat. no.
IndiSpin Pathogen Kit (50) *formerly QIAamp cador Pathogen Mini Kit (50)*	SP54104
IndiSpin Pathogen Kit (250) *formerly QIAamp cador Pathogen Mini Kit (250)*	SP54106
intype IC-DNA	IC289980
intype IC-RNA	IC289970

INDICAL offers a broad range of ready-to-use pathogen specific ELISA kits, qPCR/ RT-qPCR assays and reagents.

To optimize your workflow, and to handle your sample and throughput needs, INDICAL additionally offers instruments and kits for the efficient extraction of nucleic acids from a variety of sample types.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, IndiMag, IndiSpin, intype, pigtype and virotype products.

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Change index

Handbook	Version	Change
HB-0934-EN-003	January 2020	INDICAL design

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Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**

IndiSpin Pathogen Kit
Quick Start Protocol

For manual use.

The IndiSpin Pathogen Kits (cat. no. SP54104 and SP54106) can be stored at room temperature (15-25°C). For expiry date information, read the label on the kit box.

Further information and support

- IndiSpin Pathogen Kit Handbook: **www.indical.com/handbooks**
- Technical assistance: **support@indical.com**

Important notes before starting

- Dissolve carrier RNA in Buffer AVE as indicated on the tube.
- Add isopropanol (100%) to Buffer ACB and ethanol (96-100%) to Buffers AW1 and AW2 before use. See the respective bottle labels for volumes.
- Carry out all centrifugation steps at room temperature in a conventional table-top microcentrifuge.
- Equilibrate buffers to room temperature (15-25ºC).

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Procedure

1. Pipet 20 µl Proteinase K into a 2 ml microcentrifuge tube (not provided).
2. Add 200 µl fluid sample to the Proteinase K.

   Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS.
3. Add 100 µl Buffer VXL. Close the cap and mix by pulse vortexing.

   Note: For cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl Buffer VXL.
4. Incubate at 20-25°C for 15 min.
5. Briefly centrifuge the tube to remove drops from the inside of the lid.
6. Add 350 µl Buffer ACB to the sample and mix thoroughly by pulse-vortexing.
7. Briefly centrifuge the tube to remove drops from the inside of the lid.
8. Transfer the lysate to a spin column placed in a 2 ml collection tube. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
9. Add 600 µl Buffer AW1 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
10. Add 600 µl Buffer AW2 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
11. Centrifuge the spin column at 20,000 x *g* (14,000 rpm) for 2 min.
12. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the filtrate. Add 50-150 µl Buffer AVE to the center of the membrane, close the cap and incubate at room temperature for 1 min.
13. Centrifuge at 20,000 x *g* (14,000 rpm) for 1 min.

June 2018 | EN

intype® IC-RNA Handbook

Control RNA for real-time RT-PCR

REF 1 ml (cat. no. IC289970)

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

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Contents

Reagent contents

intype IC-RNA **Cat. no.**	**IC289970**
intype IC-RNA (ca. 8 x 10^5 copies/ µl)	1 x 1 ml
Handbook	

Intended use

intype IC-RNA is a heterologous control RNA. It can be used as amplification control to exclude false-negative results caused by PCR inhibitors and as control for RNA extraction.

Symbols


Legal manufacturer

Lot number


Use by date

Temperature limitations for storage


Handbook

Catalog number

Quality control

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

Storage

The intype IC-RNA should be stored at -30°C to -15°C and is stable until the expiration date stated on the label. Avoid repeated thawing and freezing (>5x).

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**.

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

Extraction control

The addition of intype IC-RNA to the sample that is to be extracted should be adjusted to the extraction protocol used. intype IC-RNA should be added after pipetting the lysis buffer to the sample to be extracted. In case the extraction method used includes a lysis step in combination with a heating step, it is recommended to add intype IC-RNA after the heating step. The volume of intype IC-RNA depends on the final elution volume and should be 1/10 of the elution volume (i.e. add 5 µl intype IC-RNA for 50 µl elution volume).

Amplification control

Add intype IC-RNA to the Master Mix of your RT-PCR. The appropriate volume of intype IC-RNA should be tested for each RT-PCR protocol.

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 bactotype, cador, cattletype, flocktype, pigtype and virotype products.

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Change index

Handbook	Version	Change
HB-1742-EN-002	June 2018	INDICAL design

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IndiMix™ JOE

June 2023| EN

1.5 mL (Cat. no. MX299945)
15 mL (Cat. no. MX299947)

Contents	Number of vials	Volume	Storage Conditions
Buffer, enzymes, primers, and probe to identify the intype® IC-DNA and IC-RNA Internal Control	1 (Cat. no. MX299945) 10 (Cat. no. MX299947)	1.50 mL	-30ºC to -15ºC

Description

IndiMix JOE enables real-time amplification of single or multiple nucleic acid targets and contains both reverse transcriptase and polymerase to amplify DNA and RNA targets. The control primers and probes are premixed to identify the intype IC-DNA (Cat. no. IC289980) Internal Control as well as the intype IC-RNA (Cat. no. IC289970) Internal Control. The intype Internal Controls are available separately from INDICAL. The probe targeting the intype Internal Controls is labelled as JOE™ and detected in the JOE/HEX™/VIC® channel. IndiMix JOE contains ROX™ dye as a passive reference dye for use on the Applied Biosystems™ instruments, including the Applied Biosystems 7500, ViiA™ 7 and QuantStudio™ Systems. IndiMix JOE is compatible with instrumentation that does not require ROX dye, including the Rotor-Gene® Q and Bio-Rad CFX96™. IndiMix JOE was designed to work with the fast mode or cycling parameters of various thermocyclers.

Storage and Handling

- Upon receipt, store the material at -30°C to -15ºC, secured from any sources of contaminating DNA or RNA, especially amplified DNA
- Protect from light during storage and handling
- Avoid repeated thawing and freezing (>5x) as this may reduce performance
- If used intermittently, aliquot the material and freeze
- Use aerosol barrier pipet tips for pipetting
- Do not use after the expiration date printed on the label
- Dispose of all sample residues and objects per national and local regulations

Internal Control information

The Internal Control template, usable as extraction or amplification control, is available separately as intype IC-DNA (Cat. no. IC289980) and intype IC-RNA (Cat. no. IC289970). The internal control monitors the entire PCR workflow for the presence of inhibitors or other workflow issues, including extraction, reagent or instrument errors and failures.

- To use as an extraction control, add 2-5 µL of the intype IC-DNA or intype IC-RNA per sample to the lysis buffer before nucleic acid purification
- To use as an amplification control, add 0.2-0.4 µL of the intype IC-DNA or intype IC-RNA per PCR reaction

Procedure

Table 1. Preparation of the Reaction Mix*

Component	Volume per reaction
IndiMix JOE	15 µL
Primers and Probe(s) to target(s) of interest**	2 µL
Master mix***	17 µL****
Sample nucleic acid	8 µL
Total volume of PCR reaction	**25 µL**

* Use 12 µL of IndiMix JOE for 20 µL PCR reactions and adjust the remaining components accordingly
** A primer concentration of 400 to 800 nM and a probe concentration of 200 nM per final reaction is recommended
*** For amplification control, add the appropriate intype IC-DNA/RNA volume to the master mix and adjust volumes accordingly or exceed the reaction volume slightly
**** Combine IndiMix JOE (15 µL) and Primers and Probes (2 µL) for total Master mix volume (17 µL)

Procedural Recommendations

Attention: Please protect IndiMix JOE and the prepared master mix from bright and direct light, as the probes are sensitive to light. Transparent vials and tubes are recommended to allow for visual inspection when ensuring proper mixing. A medium-speed setting for automatic or stepper dispenser pipettes is recommended to ensure accurate volume transfer.

- Thaw IndiMix JOE on ice
- Invert IndiMix JOE 5 times or until mixed thoroughly, then centrifuge briefly to remove droplets from the cap
- On ice, use a sterile tube to prepare the master mix using the volumes listed in Table 1
- Invert the prepared master mix 5-10 times or until mixed thoroughly, then centrifuge briefly to remove the droplets from the cap
- Collect the master mix and transfer it to the appropriate PCR tubes/wells, handle in a PCR cooling rack or on ice
- Add 8 µl of the sample nucleic acid to the PCR tubes/wells
- Close the tubes or seal the plate and invert 5 times or until mixed thoroughly
- Spin for 5 seconds to centrifuge the droplets to the bottom of the PCR tube/wells
- Run the thermal cycler program as indicated in Table 2

Table 2. Thermal Cycler Program*

Step	Temp.	Time	Cycles
Reverse Transcription**	50°C	10 min	1
RT inactivation / initial activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing / Extension***	60°C	30 s	

* IndiMix JOE is designed to run on the fast mode or cycling parameters of thermocyclers
** Does not affect performance with DNA targets and may be left out when a run contains only DNA
*** Fluorescence data collection

The denaturation time and the annealing/extension temperature and times can be changed per lab specific configuration. Please consult INDICAL for technical support.

Analyzing the results

Carefully examine the amplification plot, adjusting the baseline and threshold values, where required. If inhibitors are not negatively affecting the result, the target and/or IC should amplify within each PCR reaction. If both the target and IC fail to amplify in a reaction, repeating the PCR amplification with a 5X dilution of the extracted sample nucleic acid in nuclease free water is recommended. For more information, please contact INDICAL Technical Support (see below).

Quality Control

Each lot of this product was manufactured and released in accordance with INDICAL's ISO-certified Quality Management System.

Safety Information

Please consult the appropriate safety data sheets (SDSs) for this product. For more information, the SDSs are available from your regional sales manager or can be provided by emailing **compliance@indical.com**. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Change index

Handbook	Version	Change
HB-2562-EN-004	June 2023	New package size, editorial changes
HB-2562-EN-003	February 2023	Editorial changes
HB-2562-EN-002	February 2022	Product Sheet revision
HB-2562-EN-001	May 2021	Product launch

For molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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NEW! IndiMix JOE

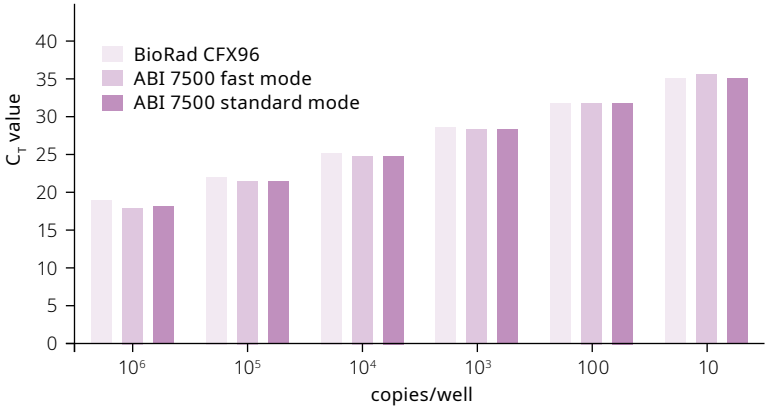
Build your assays faster and without compromise

- **Novel master mix:** Built-in, validated internal control for amplification OR extraction
- **Maximum flexibility:** One protocol for DNA and RNA applications
- **Compatible:** Works with common PCR thermocyclers
- **High-quality performance:** Inhibitor tolerant, reproducible results, even in challenging applications

One master mix. One protocol. For results you can trust.
Accelerate your assay development with our novel IndiMix JOE.

A master mix compatible with your laboratory

Figure 1



IndiMix JOE is compatible with common PCR thermocyclers and can be used to identify even the smallest number of copies per well. Get great results with both fast and standard mode cyclers.

Figure 1. A dilution series of *in vitro* RNA shows reliable performance with common PCR cyclers, down to 10 copies/well.

Extensive coverage of sample types and targets

Figure 2

Sample	DNA C_T	RNA C_T
Lung A	Negative	18.71
Lung B	7.49	Negative
Lymph node A	16.74	Negative
Serum A	20.78	Negative
Serum B	20.72	Negative
Nasal swab A	Negative	32.28
Nasal swab B	Negative	33.09

IndiMix JOE was developed and tested to work with a wide variety of animal sample matrices and applications.

INDICAL's novel IndiMix JOE master mix enables real-time amplification of single or multiple nucleic acid targets and contains both reverse transcriptase and polymerase to amplify DNA and RNA targets.

Figure 2. DNA and RNA targets from different sample types tested.

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SVANOVIR® BLV gp51-Ab

Bovine Leukaemia Virus gp51
Antibody Test
Screening format

Bovines Leukämie-Virus gp51
Antikörper Test
Monophasisch

Virus de la leucemia bovina gp51 prueba para anticuerpos
Formato de monitoreo

Test d'anticorps anti-gp51 du virus de la leucémie bovine
Format de dépistage

Contents	**Art. No. SV-104886**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious BLV gp51 antigen (sealed and stored dry)	10 (Strips) 12 x 8
Conjugate Ready-to-use (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)	1 x 120 mL
PBS-Tween Solution 20 x concentrate	3 x 125 mL
Sample Dilution Buffer Ready-to-use	1 x 120 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 100 mL
Stop Solution Contains sulphuric acid (2M) - DANGER! 	2 x 25 mL
A. Positive Control Serum - Contains preservatives	1 x 0.5 mL
B. Negative Control Serum - Contains preservatives	1 x 0.5 mL

This manual covers the following SVANOVIR® BLV gp51-Ab kit: Article number SV-104886

Bovine Leukaemia Virus gp51
Antibody Test
Screening format

Name and Application
The SVANOVIR® BLV gp51-Ab is an Enzyme Linked Immunosorbent Assay (ELISA) for the detection of antibodies against BLV gp51 in bovine serum, plasma and milk samples; individualsamples, pools and bulk tank milk.

General information
Bovine leukaemia virus (BLV) is an exogenous oncogenic retrovirus infecting B lymphocytes. The infection may lead to persistent lymphocytosis and, after lifelong latency, *i.e.* in some adult cattle, to development of tumours (lymphosarcomas) with associated symptoms[1,2]. The spread does often start from infected cattle introduced into herds and then take enzootic proportions. This has given the disease the name 'enzootic bovine leucosis' – in contrast to sporadic or juvenile bovine leucosis of unknown etiology. Since BLV is bound to lymphocytes, the exact mode of transmission by direct contact is unknown. In some countries transmission by horseflies is anticipated[3]. Transplacental transmission and transmission via milk are considered uncommon. On the contrary, iatrogenic transmission occurs easily since as little as 0.1 µL blood from an infected cow did transmit infection[4]. There is an antibody response to infection, and antibodies are found in both serum and milk. The routine diagnosis of BLV infection is thus based on antigen-antibody reaction[5]. Eradication by culling, if planned by a country with a high BLV-infection rate, is a formidable task. The control program usually starts from the results of a country-wide screening for antibodies to BLV, particularly in dairy herds.

Principle
The kit procedure is based on a solid phase indirect ELISA. A monoclonal antibody against gp51 is adsorbed to microtiter plates or strips. This antibody captures gp51 antigen. BLV antibodies (if present in the test sample) bind to the antigen in the well. HRP conjugate added subsequently forms a complex with the BLV antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of a blue colour. The reaction is stopped by addition of the stop solution; the colour changes to yellow. The result can be read visually or by a microplate photometer, where the optical density (OD) is measured at 450 nm.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled, deionised or any similar high quality water
4. Wash bottle, multichannel pipetors or plate washer
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter

Specimen information

Individual serum or pools up to 10 samples:
4 µL of blood serum or plasma is needed for each sample well. Fresh, refrigerated or previously frozen serum or plasma may be tested.

Individual or pooled milk samples/bulk tank milk up to at least 50 animals:
100 µL of skim milk is required for each sample well. Fresh, refrigerated, or previously frozen milk samples may be tested. It is recommended to centrifuge milk samples for 15 minutes at 2000 x g to remove the lipid layer, or leave the milk samples until the fat layer is formed on top of the sample. Pipette under the fat layer.

Preparation of reagents

PBS-Tween Buffer:
Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 mL per plate by adding 25 mL PBS-Tween solution to 475 mL distilled water and mix thoroughly.
N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature, 18 -25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expiry.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipette tip for each sample.
10. Do not pipette by mouth.
11. Include positive and negative control serum on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers etc., measure the required volume.
14. The Stop Solution contains sulphuric acid, which is corrosive.*
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!
There is always a surplus volume for the liquid reagent. The volume mentioned on the label is the minimum obtainable.
Strips with broken seal can be stored 2-8°C (36-46°F) for up to 4 weeks.

Procedure

1. All reagents should equilibrate to room temperature 18-25˚C (64-77˚F) before use. Label each strip with a number.
2. Add samples

   The provided negative and positive control sera are used for both serum and milk testing.

   Serum Samples

A. Add 100 µL of Sample Dilution Buffer to each well that will be used for serum samples and serum controls.

B. Add 4 µL of Positive Control Serum (Reagent A) and 4 µL of Negative Control Serum (Reagent B) respectively to selected wells coated with BLV gp51 viral antigen.

   For confirmation purposes it is recommended to run the samples in duplicates.

C. Add 4 µL of serum sample to a selected well coated with BLV gp51 viral antigen.The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

   Continue at step #3

   Milk Samples

A. For addition of controls, see "serum Samples" (point A and B).

B. Add 100 µL of milk sample to a selected well coated with BLV gp51 viral antigen. The samples can be tested in singlicates or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

   Continue at step #3

3. Shake the plate thoroughly. Seal the plate/strip and incubate at 37˚C (98.6˚F) for 1 hour.
4. Rinse the plates/strips 3 times with PBS-Tween Buffer: At each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
5. Add 100 µL of HRP Conjugate to each well and incubate at 37˚C (98.6˚F) for 1 hour.
6. Repeat step #4.
7. Add 100 µL Substrate Solution to each well. Incubate for 10 minutes at room temperature, 18-25˚C (64-77˚F). Begin timing when the first well is filled.
8. Stop the reaction by adding 50 µL of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #7.
9. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank).

   Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculations

Calculations of results are done as described below.

Calculation of Percent Positivity Values (PP)

All OD Values for the test samples as well as the Negative Control are related to the OD value of the positive control as follows:

$$PP = \frac{OD_{\text{Sample or Negative control}}}{OD_{\text{Positive control}}} \text{ X100}$$

Interpretation of the results

Criteria for test validity

TTo ensure validity, the duplicate OD values of the positive control should not differ more than 25% from the mean value of the two duplicates.
Additionally, the control values should fall within the following limits:

OD Positive Control	**> 1.0**
PP Negative Control	**< 15**

Should any of these criteria not be fulfilled, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.
To confirm test sample results, each separate duplicate PP values should be equally interpretated, *i.e.* positive and negative. In case of discrepancy it is recommended to re-test the sample.

Interpretation of test sample result

Sample	PP	Interpretation
Serum	< 20 ≥ 20	Negative Positive
Milk	< 15 ≥ 15	Negative Positive

References

1. Burny, A., Bruck, C. *et al*. (1980) Bovine leukemia virus: molecular biology and epidemiology. In Viral Oncology. Edited by G. Klein. New York, Raven Press. 231-289.
2. Ferrer, J.,F. (1980) Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med. 24, 1-68.
3. Manet, G. *et al*. (1989). Natural mode of horizontal transmission of bovine leukemia virus (BLV): the potential role of tabanids (*Tabanus spp*.). Vet. Immunol. Immunopathol. 22, 255-263.
4. Evermann, J.F. *et al*. (1986). Transmission of bovine leucosis virus by blood inoculation. Am. J. Vet. Res. 47, 1885-1887.
5. Hoff-Joergensen, R. (1989). An international comparision of different laboratory tests for the diagnosis of bovine leucosis: suggestions for international standardization. Vet. Immunol. Immunopathol. 22, 293-297.

***DANGER: Stop solution (sulphuric acid)**

May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.

IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.

IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

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Zusammensetsung	Artikelnummer SV-104886
Mikrotiterplatten Mikrotiterplatten (96 Vertiefungen) beschichtet mit nicht-infektiösem BLV gp51-Antigen (versiegelt und trocken)	10 (Streifen) 12 x 8
Konjugat Gebrauchsfertig (Meerrettichperoxidase-konjugierte anti-Rind IgG monoklonale Antikörper) (jede Flasche 11,5 ml nach Auflösen)	1 x 120 ml
PBS-Tween-Lösung 20-fach konzentriert	3 x 125 ml
Probenverdünnungspuffer Gebrauchsfertig	1 x 120 ml
Substratlösung (Tetramethylbenzidin in Substratpuffern mit H_2O_2) - IM DUNKELN AUFBEWAHREN!	1 x 100 ml
Stopplösung Enthält Schwefelsäure (2M) - GEFAHR! 	2 x 25 ml
A. Positives Kontrollserum - Enthält ein Konservierungsmittel	1 x 0,5 ml
B. Negatives Kontrollserum - Enthält ein Konservierungsmittel	1 x 0,5 ml

Diese Gebrauchsinformation umfasst den SVANOVIR® BLV gp51 ELISA mit der Artikelnummern SV-104886

Bovines Leukämie-Virus gp51
Antikörper Test
Monophasisch

GEBRAUCHSINFORMATION; nach §11 Absatz 2
TierGesG zugelassen; Zul.Nr.:FLI-C 137

Name und Anwendung
Der SVANOVIR® BLV gp51-Ab ist ein Enzymgekoppelter Immuno-Assay (ELISA) zum Nachweis von Antikörpern gegen BLV gp51 in Blutserum, -plasma und in Milch; Einzel und Sammelproben (inkl. Tankmilch) von Rindern.

Allgemeine Information
Das bovine Leukämie-Virus (BLV) ist ein exogenes, onkogenes Retrovirus, das die B-Lymphozyten befällt. Die Infektion kann zu einer persistenten Lymphozytose führen und nach lebenslanger Latenz bei manchen adulten Rindern die Entwicklung von Tumoren (Lymphosarkome)mit entsprechen-den Symptomen[1,2] zur Folge haben. Die Ausbreitung erfolgt oftmals über ein in die Herde integriertes infiziertes Rind und nimmt anschließend enzootische Ausmaße an. Daraus leitet sich der Name der Krankheit 'enzootische bovine Leukose' ab – im Gegensatz zur sporadischen bovinen Leukose unbekannter Ätiologie. Da BLV die Lymphozyten befällt ist der genaue Übertragungsmodus bei direktem Kontakt unbekannt. In manchen Ländern wird eine Übertragung durch Pferdebremsen diskutiert[3]. Eine transplazentare Übertragung sowie die Übertragung über Milch sind selten. Auf der anderen Seite erfolgt die iatrogene Übertragung sehr leicht, da lediglich 0,1 µl Blut eines infizierten Rindes nötig sind, um eine Infektion zu verursachen[4]. Die Infektion löst eine Antikörperreaktion aus und diese Antikörper sind sowohl im[5]Serum als auch in der Milch zu finden. Die Routine-Diagnose einer BLV-Infektion beruht daher auf der Antigen-Antikörper-Reaktion. Die Ausrottung durch Keulung in Ländern mit hoher BLV-Infektionsrate ist ein gewaltiges Unterfangen.
Das Kontrollprogramm beginnt üblicherweise mit dem Vorliegen der Resultate eines landesweiten Screening-Programms für Antikörper gegen BLV, vor allem in Milchviehherden.

Diagnostisches Verfahren
Der bovine Leukämie-Virus-Antikörper-Test ist ein indirekter ELISA zum spezifischen Nachweis von Antikörpern gegen BLV-51 kDalton Glykoprotein (gp51) in Serum-, Plasma- und Milchproben. Ein monoklonaler Antikörper gegen gp51 wird auf Mikrotiterplatten oder -streifen aufgebracht. Dieser Antikörper reagiert mit dem gp51-Antigen. Die BLV-Antikörper (so in der Probe vorhanden) binden an das Antigen in der Vertiefung. Das anschließend zugegebene HRP-Konjugat bildet mit den BLV-Antikörpern einen Komplex. Ungebundenes Material wird vor der Zugabe einer Substratlösung durch Waschvorgänge entfernt. Anschließend entwickelt sich aufgrund der Umwandlung des Substrats durch das Konjugat blaue Farbe, die ein positives Resultat anzeigt. Durch die Zugabe einer Stopp-Lösung wird die Reaktion beendet. Die Farbe ändert sich zu gelb. Das Ergebnis kann visuell oder mit einem Photometer für Mikrotiterplatten gelesen werden, wobei die optische Dichte (OD) bei 450 nm gemessen wird.

Zusätzlich notwendiges Material

1. Präzisionspipetten
2. Einmalpipettenspitzen
3. Destilliertes, deionisiertes Wasser oder Wasser mit ähnlichem Qualität
4. Einrichtung zum Aufbringen und Absaugen der Waschlösung
5. Ein Behälter für die PBS-Tween-Puffer (1 bis 2 Liter)
6. Photometer für die Mikrotiterplatten mit 450 nm Filter

Probenvorbereitung

Serum: Einzelproben oder Sammelproben mit bis zu 10 Proben:

Für jede Probenvertiefung werden 4 µl Blutserum oder Plasma benötigt. Für den Test geeignet sind frische, gekühlte oder aufgetaute Serum- oder Plasmaproben.

Milch: Einzel- oder Sammelproben von bis zu 50 Tiere:

Für jede Probenvertiefung werden 100 µl entrahmter Milch benötigt. Für den Test geeignet sind frische, gekühlte oder aufgetaute Milchproben. Es wird empfohlen, die Milchproben 15 Minuten bei 2000 x g zu zentrifugieren, um die Fettschicht zu entfernen oder die Milchproben solange stehen zu lassen, bis sich die Fettschicht über der Probe abgelagert hat. Unter der Fettschicht pipettieren.

Zubereitung der Reagenzien

PBS-Tween-Puffer:

Für die Bearbeitung einer Mikrotiterplatte verdünnen Sie 25 ml der 20-fach konzentrierten PBS-Tween-Lösung mit 475 ml destilliertem Wasser. Mischen Sie sorgfältig!

Anmerkung: Überprüfen sie, ob sich in der Flasche kristalliner Niederschlag befindet. In diesem Fall bitte erwärmen und gut schütteln.

Besondere Hinweise

1. Alle Hinweise vor der Testdurchfürung sorgfältig lesen und befolgen.
2. Das Test-Kit und alle Reagenzien bei 2-8˚C lagern.
3. Alle Reagenzien vor Gebrauch auf Zimmer-temperatur, (18-25˚C) bringen.
4. Alle Materialen entsprechend den Richtlinien der guten Laborpraxis behandeln.
5. Nicht die Reagenzien und/oder Anweisungen verschiedener Tests untereinander vertauschen.
6. Kontamination der Testreagenzien verhindern.
7. Test nach Ablauf der Haltbarkeit nicht mehr verwenden.
8. Während der Testdurchführung nicht essen, trinken oder rauchen.
9. Für jede Probe eine separate Pipettenspitze benutzen.
10. Nicht mit dem Mund pipettieren.
11. Bei jeder Testdurchführung muß eine positive und negative Kontrolle mitgeführt werden.
12. Ausschließlich destilliertes Wasser oder Wasser mit ähnlich hoher Qualität zur Herstellung der Testreagenzien verwenden.
13. Wenn Sie die verschiedenen Gebrauchslösungen herstellen, bitte das benötigte Volumen genau abmessen, da bei den flüssigen Reagenzien mehr Volumen als angegeben abgefüllt wurde.
14. Die Stopplösung enthält Schwefelsäure und ist daher ätzend.*
15. Alle nicht verwendeten biologischen Materialien entsprechend den lokalen, regionalen und nationalen Bestimmungen entsorgen.

Hinweise

Die Angabe auf dem Etikett entspricht der minimal erhältlichen Menge.
Streifen mit beschädigter Abdeckung können bei 2-8˚C bis zu 4 Wochen gelagert werden.

Durchführung des Tests

1. Vor der Testdurchführung sämtliche Reagenzien auf Raumtemperatur (18-25°C) bringen. Jeden Streifen mit einer Nummer versehen.
2. Proben zugeben.

   Das negative bzw. positive Kontrollserum wird auch für die Testdurchführung bei Milchproben benutzt.

   Serumproben

A. 100 µl Probenverdünnungspuffer in jede für Serumproben und Serumkontrollen vorgesehene Vertiefung geben.

B. 4 µl positives Kontrollserum (Reagenz A) beziehungsweise 4 µl negatives Kontrollserum (Reagenz B) in die mit BLV gp51-Antigen beschichteten Vertiefungen geben.

   Zur Bestätigung wird empfohlen, die Kontrollseren im Doppelansatz laufen zu lassen.

C. 4 µl Serumprobe in eine mit BLV gp51-Antigen beschichtete Vertiefung geben. Die Proben können entweder einzeln oder im Doppelansatz getestet werden. Zur Sicherheit wird jedoch empfohlen, eine Doppel-bestimmung der Proben vorzunehmen.

   Fahren Sie mit weiteren Durchführung des Tests fort wie ab Schritt 3 beschrieben.

   Milchproben

A. Für die Zugabe der Kontrollen siehe unter "Serumproben" (Punkt A und B)

B. 100 µl Milchprobe in eine mit BLV gp51-Antigen beschichtete Vertiefung geben. Die Proben können entweder einzeln oder im Doppelansatz getestet werden. Zur Sicherheit wird jedoch empfohlen, eine Doppelbestimmung der Proben vorzunehmen.

   Fahren Sie mit weiteren Durchführung des Tests fort wie ab Schritt 3 beschrieben.

3. Die Platte gründlich mischen. Die Platte/den Streifen versiegeln und für 1 Stunde bei 37°C inkubieren.
4. Die Platten/Streifen 3 Mal mit PBS-Tween-Puffer spülen: Bei jedem Spülvorgang die Vertiefungen anfüllen, die Platten entleeren und die verbleibende Flüssigkeit gründlich entfernen.
5. In jede Vertiefung 100 µl Konjugat geben und 1 Stunde bei 37°C inkubieren.
6. Schritt 4 wiederholen.
7. 100 µl Substratlösung in jede Vertiefung geben und 10 Minuten bei Raumtemperatur, (18-25°C) inkubieren. Die Zeitmessung beginnt mit dem Befüllen der ersten Vertiefung.
8. Durch Zugabe von 50 µl Stopplösung und sorgfältiges Vermischen die Reaktion beenden. Die Stopplösung auf gleiche Weise zugeben wie in Schritt 7 für die Substratlösung beschrieben.
9. Die Extinktionswerte (OD-Werte) der Kontrollen und Proben bei 450 nm mit einem Photometer für Mikrotiterplatten messen (Luft als Leerwert nehmen).

   Die Extinktionswerte innerhalb von 15 Minuten nach Zugabe der Stopplösung messen, um Fluktuationen der Werte zu vermeiden.

Auswertung

Die Auswertung der Ergebnisse erfolgt wie im Folgenden beschrieben:

Prozentuale Probenwerte (PP)

Diese werden errechnet, indem man alle OD-Werte der Proben und der negativen Kontrollen mit den OD Werten der positiven Kontrollen entsprechend folgender Formel in Beziehung setzt:

$$PP = \frac{OD_{\text{der Probe/des negative Kontrollserum}}}{OD_{\text{der positiven Kontrollserum}}} X100$$

Interpretation der Ergebnisse

Validitätskriterien

Um die Validität zu gewährleisten, sollte der OD-Wert der einzelnen Ergebnisse des Doppelansatzes vom positiven Kontrollserum um nicht mehr als 25 % vom Mittelwert des Doppelansatzes abweichen. Die Kontrollwerte sollten sich innerhalb folgender Grenzen bewegen:

OD	**Positive Kontrolle**	**> 1,0**
PP	**Negative Kontrolle**	**< 15**

Wird eines dieser Kriterien nicht erfüllt, ist der Test nicht gültig. Bei nicht gültigen Tests könnte ein Fehler aufgetreten sein und der Test sollte wiederholt werden.
Zur Bestätigung der Ergebnisse sollten die PP-Werte des Doppelansatzes einzeln ausgewertet werden und ein gleichermaßen positives und negatives Ergebnis aufweisen. Im Falle einer Diskrepanz wird eine erneute Untersuchung der Probe empfohlen.

Beurteilung der Probenergebnisse

Probe	PP	Beurteilung
Serum	< 20 ≥ 20	Negativ Positiv
Milch	< 15 ≥ 15	Negativ Positiv

Referenzen

1. Burny, A., Bruck, C. *et al*. (1980) Bovine leukemia virus: molecular biology and epidemiology. In Viral Oncology. Edited by G. Klein. New York, Raven Press. 231-289.
2. Ferrer, J.,F. (1980) Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med. 24, 1-68.
3. Manet, G. *et al*. (1989). Natural mode of horizontal transmission of bovine leukemia virus (BLV): the potential role of tabanids (*Tabanus spp*.). Vet. Immunol. Immunopathol. 22, 255-263.
4. Evermann, J.F. *et al*. (1986). Transmission of bovine leucosis virus by blood inoculation. Am. J. Vet. Res. 47, 1885-1887.
5. Hoff-Joergensen, R. (1989). An international comparision of different laboratory tests for the diagnosis of bovine leucosis: suggestions for international standardization. Vet. Immunol. Immunopathol. 22, 293-297.

***GEFAHR: Stopplösung (Schwefelsäure)**
Kann gegenüber Metallen korrosiv sein. Verursacht Hautreizungen. Verursacht schwere Augenreizung. Nur im Originalbehälter aufbewahren. Schutzhandschuhe/Schutzkleidung/ Augenschutz/Gesichtsschutz tragen. BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen. Sofort GIFTINFORMATIONSZENTRUM/ Arzt/ anrufen. Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.
BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/waschen.
Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen. Bei Hautreizung: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen. Verschüttete Mengen aufnehmen, um Materialschäden zu vermeiden.

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Contenido	Nº de artículo SV-104886
Microplacas Microplacas (96 pocillos) recubiertas con antígeno de BLV gp51 no infeccioso (selladas y almacenadas en seco)	10 (Tiras) 12 x 8
Conjugado Listo para usar (peroxidasa de rábano conjugado con anticuerpos monoclonales IgG anti-bovinos)	1 x 120 ml
Solución PBS-Tween 20x concentrado	3 x 125 ml
Tampón para dilución de muestra Listo para usar	1 x 120 ml
Solución substrato (Tetrametilbenzidina en tampón de substrato con H_2O_2) - ALMACENAR EN OBSCURO¡	1 x 100 ml
Solución frenadore Contiene ácido sulfúrico (2M) - PELIGRO¡ 	2 x 25 ml
A. Suero control positivo - Contiene conservante	1 x 0,5 ml
B. Suero control negativo - Contiene conservante	1 x 0,5 ml

Manual de kit SVANOVIR® BLV gp51-Ab: N° articulo: SV-104886

Virus de la leucemia bovina gp51 prueba para anticuerpos

Formato de monitoreo

Nombre y aplicación

SVANOVIR® BLV gp51-Ab es un inmunoensayo (ELISA) para la detección de anticuerpos contra BLV gp51 en suero bovino, plasma y leche; muestra individuale, mezcla de muestra o leche y en tanques de leche.

Información general

El virus de la leucemia bovina (BLV) es un retrovirus oncogénico exógeno que infecta los linfocitos B. La infección puede causar linfocitosis persistente o no y, tras permanecer latente toda la vida, por ejemplo en algunos animales adultos, desarrollar tumores (linfosarcomas) y síntomas asociados[1,2]. A menudo, la propagación comienza cuando entra ganado infectado en el rebaño y después toma proporciones enzoóticas. Por ello se ha llamado a la enfermedad "leucosis bovina enzoótica", como contraste a la leucosis bovina esporádica o juvenil de etiología desconocida. Debido a que BLV está asociado a los linfocitos, se desconoce el mecanismo exacto de transmisión por contacto directo. En algunos países viene precedida por la transmisión por tábanos[3]. La transmisión trasplacentaria y por leche se consideran poco comunes. Por el contrario, la transmisión iatrogénica se produce fácilmente, ya que la enfermedad se contagia con tan solo 0,1 µl de sangre procedente de una vaca infectada[4]. Los anticuerpos responden a la infección, y se encuentran en el suero y en la leche. Por tanto, el diagnóstico rutinario de la infección por BLV se basa en una reacción antígeno-anticuerpo[5]. La erradicación de la enfermedad por sacrificios selectivos en un país con una alto índice de infección por BLV es una tarea muy compleja. El programa de control comienza normalmente a partir de los resultados de un monitoreo serológico a nivel nacional, en particular en hatos productores de leche.

Principio

El procedimiento del kit se basa en un ELISA indirecto en dónde anticuerpos monoclonales contra gp51 están inmobilizados en pocillos de microplacas o tiras. Los anticuerpos capturan los antígenos gp51del medio de cultivo celular infectado con BLV. Los anticuerpos de BLV (si están presentes en la muestra) se fijan al antígeno en los pocillos. Subsequentemente, se añade el conjugado de peroxidasa de rábano (HRP) que forma un complejo con los anticuerpos contra BLV. El material libre se elimina lavando los pocillos antes de añadir la solución substrato. Subsequentemente, aparece un color azul debido a la conversión del substrato por parte del conjugado. El resultado positivo se indica con la aparición de un color azulado. La reacción se detiene con la adición de la solución frenadora y el color cambiará a amarillo. El resultado puede leerse por medio de un fotómetro de microplacas, en el que se mide la densidad óptica (DO) a 450 nm.

Materialales necesarios (no suministrados)

1. Pipetas de precisión
2. Puntas de pipetas desechables
3. Agua destilada, deionizada o cualquier otra agua altamente purificada
4. Botella para enjuague, sistema de enjuague de pipetas o placas multicanales
5. Recipiente: de 1 a 2 litros para PBS-Tween
6. Fotómetro para microplacas, filtro de 450 nm

Información de las muestras

Suero individual o mezcla de hasta 10 muestras:

Se necesitan 4 µl de suero sanguíneo o plasma para cada pocillo /muestra. La prueba puede realizarse con suero o plasma recién obtenido, refrigerado o congelado previa-mente.

Leche individual o conjunto de muestras de leche hasta de 50 animales:

Se requieren 100 µl de leche desnatada para cada pocillo/muestra. La prueba puede realizarse con leche recién obtenidos, refrigerados o congelados previamente.
Se recomienda centrifugar las muestras de leche durante 15 minutos a 2000 x g para retirar la capa de grasa (lípidos), o dejar reposar las muestras de leche hasta que se forme una capa de grasa en la superficie. Pipetar debajo de la capa de grasa.

Preparación de los reactivos

Tampón PBS-Tween:

Diluir la solución PBS-Tween 20 x en una proporción de 1/20 en agua destilada. Preparar 500 ml por placa añadiendo 25 ml de solución PBS-Tween a 475 ml de agua destilada y mezclar muy bien.
Nota: comprobar que no se ha producido precipitación de cristales en la botella.
Si se observan cristales, la solución debe calentarse y agitarse bien.

Precauciónes

1. Leer con atención y seguir todas las instrucciones.
2. Conservar el kit y todos los reactivos de 2-8°C.
3. Antes de su uso, debe dejarse que los reactivos alcancen temperatura ambiente de 18-25°C .
4. Manipular todos los reactivos observando las Buenas Prácticas de Laboratorio (GLP).
5. No mezclar componentes o manuales de instrucciones de kits de distintos lotes.
6. No contaminar los componentes del kit.
7. No utilizar el kit pasada la fecha de caducidad.
8. No comer, beber o fumar cuando se manipulen las muestras o los reactivos del kit.
9. Utilizar puntas de pipetas distintas para cada muestra.
10. No usar la pipeta con la boca.
11. Incluir controles positivos y negativos de suero en cada serie de placas o tiras.
12. Para la preparación de los reactivos, utilizar únicamente agua destilade, deionisada o cualquier otra agua altamente purificada.
13. Cuando prepare la solucion tampón etc. medir el volúmen requerido.
14. La solución frenadora contiene ácido sulfúrico que es muy corrosivo.*
15. Los materiales biológicos no utilizados deben desecharse siguiendo las normativas locales, regionales o nacionales.

Recomendaciones

Los reactivos liquidos, se envian siempre en volúmenes algo mayores. El volumen mencionado en la etiqueta de los frascos es el minimo que se puede obtener.
Las tiras con sellado abierto pueden almacenarse a una temperatura de 2-8°C hasta 4 semanas.

Procedimiento

1. Antes de su uso, debe dejarse que los reactivos alcancen una temperatura ambiente, 18-25°C. Marcar cada tira con un número.
2. Añadir las muestras.

   Los sueros controles negativo y positivo incluidos en el kit, se utilizan tanto para muestra de suero come de leche.

   Muestras de suero

A. Añadir 100 µl de tampón para dilución de muestras a cada pocillo que use para las muestras de suero controles.

B. Añadir 4 µl de suero control positivo (Reactivo A) y 4 µl de suero control negativo (Reactivo B) respectivamente a los pocillos recubiertos con antígeno BLV gp51. Para confirmación, se recomienda correr los controles de suero por duplicado.

C. Añadir 4 µl de la muestra de suero a un pocillo recubierto con antígeno BLV gp51.

   Las muestras pueden ser procesadas individualmente o en duplicado. Sinembargo para propósito de confirmación se recomienda correr las muestras en duplicado.

   Continuar con el paso #3.

   Muestras de leche

A. Para la adición del control positivo y control negativo ver "Muestras de suero" (paso A y B).

B. Añadir 100 µl de muestra de leche a un pocillo recubierto con antígeno BLV gp51.

   Las muestras pueden ser procesadas individualmente o en duplicado. Sinembargo para propósito de confirmación se recomienda correr las muestras en duplicado.

   Continuar con el paso #3.

3. Agitar bien la placa. Sellar la placa /tira e incubar a 37°C durante 1 hora.
4. Enjuagar las placas/tiras 3 veces con tampon PBS-Tween: en cada ciclo de enjuague, rellene los pocillos, vacíe la placa y golpéela sobre una superficie cubierta con material absorbente para eliminar todo resto de líquido.
5. Añadir 100 µl de conjugado HRP a cada pocillo. Sellar la placa /tira e incubar a 37°C durante 1 hora.
6. Repetir el paso # 4.
7. Añadir 100 µl de la solución substrato a cada pocillo. Incubar durante 10 minutos a temperatura ambiente, 18-25°C. Comience a cronometrar al llenar el primer pocillo.
8. Interrumpir la reacción añadiendo 50 µl de solución frenadora a cada pocillo y mezclar bien. Añadir la solución frenadora en el mismo orden en que llenó con solución de substrato en el paso # 7.
9. Medir la densidad óptica (DO) de los controles y las muestras a 450 nm con un fotómetro para microplacas (aire como muestra en blanco).

   Medir la DO en el intervalo de 15 minutos tras haber añadido la solución frenadora para evitar fluctuaciones en los valores de DO.

Cálculos

Los cálculos de resultados se realizan de la siguiente forma:

Valores positivos porcentuales (PP)

Todos los valores de DO de las muestras y controles negativos se relacionan con el valor de DO del control positivo de la siguiente forma:

$$PP = \frac{DO_{\text{Muestra o Control negativo}}}{DO_{\text{Control positivo}}} X100$$

Interpretación de los reultados

Interpretación de los reultados de la muestras

Para garantizar la validéz, los valores duplicados de DO del control positivo no deben diferir en más de un 25% del valor medio de los dos duplicados.
Además, los valores de control (de suero y leche) deben encontrarse entre los límites siguientes:

DO Control positivo	**> 1,0**
PP Control negativo	**< 15**

Si no se cumple alguno de estos criterios, la prueba no se considerará válida. Si la prueba no es válida, es probable que se deba a la técnica empleada y la prueba debe repetirse. Para confirmar los resultados de la prueba, deben interpretarse por igual y por separado los valores duplicados de PP positivos y negativos. En caso de discrepancia, se recomienda repetir la prueba.

Interpretación de la muestras

Muestra	PP	Interpretación
Suero	< 20 ≥ 20	Negativo Positivo
Leche	< 15 ≥ 15	Negativo Positivo

Referencias

1. Burny, A., Bruck, C. *et al*. (1980) Bovine leukemia virus: molecular biology and epidemiology. In Viral Oncology. Edited by G. Klein. New York, Raven Press. 231-289.
2. Ferrer, J.,F. (1980) Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med. 24, 1-68.
3. Manet, G. *et al*. (1989). Natural mode of horizontal transmission of bovine leukemia virus (BLV): the potential role of tabanids (*Tabanus spp*.). Vet. Immunol. Immunopathol. 22, 255-263.
4. Evermann, J.F. *et al*. (1986). Transmission of bovine leucosis virus by blood inoculation. Am. J. Vet. Res. 47, 1885-1887.
5. Hoff-Joergensen, R. (1989). An international comparision of different laboratory tests for the diagnosis of bovine leucosis: suggestions for international standardization. Vet. Immunol. Immunopathol. 22, 293-297.

***PELIGRO: Solution frenadora (ácido sulfúrico)**
Puede ser corrosivo para los metales. Provoca irritación cutánea. Provoca irritación ocular grave. Conservar únicamente en el recipiente original. Llevar guantes/prendas/gafas/máscara de protección.
EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando. Llamar inmediatamente a un CENTRO DE TOXICOLOGÍA/médico. Si persiste la irritación ocular: consultar a un médico.
EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante agua y jabón. Quitar las prendas contaminadas y lavarlas antes de volver a usarlas. En caso de irritación cutánea: consultar a un médico. Absorber el vertido para que no dañe otros materiales.

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Contenu	**Nº d'article SV-104886**
Microplaques Microplaques (96 puits) sensibilisés avec l'antigène non infectieux de BLV gp51 (scellées et gardées au sec)	10 (Barrettes) 12 x 8
Conjugué Prêt à l'emploi (peroxydase de raifort conjugué à des anticorps monoclonaux IgG antibovins)	1 x 120 mL
Solution de PBS Tween Concentrée 20 fois	3 x 125 mL
Tampon de dilution des échantillons Prêt à l'emploi	1 x 120 mL
Solution de substrat (Tétraméthylbenzidine dans le tampon de substrat contenant H_2O_2) - CONSERVER Á L'OBSCURITÉ!	1 x 100 mL
Solution d'arrêt Contient de l'acide sulfurique (2M) - DANGER! 	2 x 25 mL
A. Sérum de contrôle positif - Contient des agents conservateurs	1 x 0,5 mL
B. Sérum de contrôle négatif - Contient des agents conservateurs	1 x 0,5 mL

Ce manuel concerne la trousse SVANOVIR® BLV gp51-Ab: N° d'article SV-104886

Test d'anticorps anti-gp51 du virus de la leucémie bovine

Format de dépistage

Nom et application

SVANOVIR® BLV gp51-Ab est une trousse de détection immunoenzymatique des anticorps spécifiques de la glucoprotéine gp51 du BLV dans le sérum, le plasma bovins et le lait individuel et le lait provenant de cuves.

Information générale

Le virus de la leucémie bovine (BLV) est un rétrovirus exogène oncogène infectant les lymphocytes B. L'infection peut (ou pas) conduire à une lymphocytose persistante et, après une latence durant toute la vie, c'est-à-dire chez certains bovins adultes, au développement de tumeurs (lymphosarcomes) avec symptômes associés[1,2]. La contamination commence souvent par du bétail infecté introduit dans des troupeaux et prend ensuite des proportions enzootiques. Cette caractéristique a donné à la maladie le nom de « leucose bovine enzootique » – par opposition à la leucose bovine juvénile ou sporadique d'origine inconnue. Comme le BLV est lié aux lymphocytes, le mode exact de transmission par contact direct est inconnu. Dans certains pays, une transmission par les taons est anticipée[3]. La transmission transplacentaire et la transmission par le lait sont considérées comme peu fréquentes. Au contraire, une transmission iatrogène se produit facilement puisqu'une quantité aussi faible que 0,1 µL de sang provenant d'une vache infectée a suffi pour transmettre l'infection[4]. On observe une production d'anticorps en réponse à l'infection, et des anticorps sont présents dans le lait et le sérum. Le diagnostic général de l'infection à BLV repose donc sur une réaction anticorps-antigène[5]. L'éradication par la réforme des animaux, si elle est programmée par un pays atteint d'un taux élevé d'infection à BLV, est une tâche de grande ampleur. Le projet d'éradication est lancé généralement à partir des résultats d'un dépistage national d'anticorps anti-BLV, en particulier dans les cheptels laitiers.

Principe

La méthode du kit repose sur un test en phase solide ELISA indirect. Un anticorps monoclonal anti-gp51 est adsorbé sur des plaques ou des barrettes de microtitration. Cet anticorps capture l'antigène gp51 dans le milieu de culture de cellules de BLV. Les anticorps anti-BLV (s'ils sont présents dans l'échantillon test) se lient à l'antigène dans le puits. Le conjugué HRP ajouté ensuite forme un complexe avec ces anticorps anti-BLV. Le matériel non lié est éliminé par rinçage avant l'ajout de solution de substrat. Ensuite apparaît une couleur bleue due à la conversion du substrat par le conjugué. Un résultat positif est indiqué par l'apparition d'une couleur bleue. La réaction est arrêtée par l'addition de solution d'arrêt; la couleur devient jaune. Le résultat peut être lu visuellement ou à l'aide d'un photomètre à microplaques, avec lequel la densité optique (DO) est mesurée à 450 nm.

Matériels nécessaires mais non fournis

1. Micropipettes de précision
2. Embouts jetables pour micropipette
3. Eau distillée, desionisée ou de l'eau ultrapure
4. Pissette, pipetteur à canaux multiples ou laveur de microplaques.
5. Récipient de 1 à 2 litres pour le PBS Tween
6. Photomètre à microplaques (filtre de 450 nm)

Information sur les échantillons

Sérum individuel ou mélanges jusqu'à 10 échantillons:

4 µL de sérum sanguin ou de plasma sont nécessaires pour chaque puits. Du plasma ou du sérum frais, réfrigéré ou congelé sont utilisables.

Lait individuel ou échantillons de mélanges/ lait de tank jusqu'à 50 animaux:

100 µL de lait écrémé sont nécessaires pour chaque puits. Il est recommandé de centrifuger les échantillons de lait pendant 15 minutes à 2000 x g pour éliminer la couche lipidique, ou de laisser reposer les échantillons de lait jusqu'à ce qu'une couche de graisse se forme à la surface de l'échantillon. Pipeter sous la couche de graisse.

Préparation des réactifs

Tampon PBS-Tween:

Diluer 1/20 la solution concentrée de PBS-Tween dans de l'eau purifiée. Préparer 500 mL par plaque en diluant 25 mL dans 475 mL d'eau et bien mélanger.
N.B. Avant la dilution, s'assurer qu'il ne reste pas de cristaux dans le tampon. Pour dissoudre les restes de cristaux, réchauffer et bien mélanger.

Précautions

1. Lire attentivement les instructions et s'y conformer strictement.
2. Conserver la trousse et tous les réactifs entre 2-8°C.
3. Laisser les réactifs atteindre la température ambiante entre 18-25°C avant usage.
4. Manipuler tout le matériel conformément aux bonnes pratiques de laboratoire.
5. Ne pas mélanger des composantes ni confondre des monographies de différentes séries de trousses.
6. Prendre soin d'éviter toute contamination des composantes de la trousse.
7. Respecter la date de péremption de la trousse.
8. Ne pas manger, boire, ni fumer là où sont manipulés les échantillons et les réactifs.
9. Changer d'embout de micropipette pour chaque échantillon.
10. Ne jamais pipeter à la bouche.
11. Inclure des contrôles de sérum négatifs et positifs sur chaque plaque ou série de barrettes.
12. N'utiliser que de l'eau distillée, désionisée ou ultrapure pour la préparation des réactifs.
13. Cuando prepare la solucion tampón etc. medir el volúmen requerido.
14. La solution d'arrêt contient de l'acide sulfurique, qui est corrosif.*
15. L'élimination des matériaux biologiques non utilisés doit être réalisée dans le respect des réglementations locales, régionales et nationales.

Recommandations!
Il ya toujours un surplus de volume pour le réactif liquide. Le volume mentionné sur l'étiquette est le minimum à obtenir.
Les barrettes dont l'emballage est ouvert peuvent être conservées entre 2-8°C pendant 4 semaines au maximum.

Protocole

1. Tous les réactifs doivent être portés à la température ambiante, entre 18-25°C, avant utilisation. Étiqueter chaque barrette en lui affectant un numéro.
2. Préparer les échantillons.

   Les sérums contrôles positifs et négatifs fournis seront utilisés pour la détection d'anticorps aussi bien au niveau du sérum qu'au niveau du lait.

   Échantillons de sérum

A. Déposer 100 µL de tampon de dilution des échantillons dans chaque puits qui servira pou- les échantillons de sérum et les contrôles de sérum.

B. Déposer 4 µL de sérum de contrôle positif (réactif A) et 4 µL de sérum de contrôle négatif (réactif B) dans des puits sélectionnés recouverts d'antigène viral de BLV gp51. Pour une confirmation, il est recommandé de préparer les sérums de contrôle en double.

C. Déposer 4 µL d'échantillon de sérum dans un puits sélectionné recouvert d'antigène viral de BLV gp51. Pour une confirmation, il est recommandé de préparer les échantillons en double.

   Poursuivre à l'étape 3.

   Échantillons de lait

A. Déposer 100 µL de tampon de dilution des échantillons dans chaque puits qui servira poules des contrôles de sérum.

B. Déposer 4 µL de sérum de contrôle positif (réactif A) et 4 µL de sérum de contrôle négatif (réactif B) dans des puits sélectionnés recouverts d'antigène viral de BLV gp51.

   Poursuivre à l'étape 3.

   Pour une procédure alternative:
   Pré diluer le sérum de contrôle positif (réactif A) et le sérum de contrôle negatif (réactif B) dans du tampon de dilution des échantillons à concentration 1:25 (exemple 20 µL de sérum de contrôle dans 500 µL tampon de dilution des échantillons). Déposer respectivement 100 µL du réactif A et 100 µL du réactif B aux puits correspondants.

   Pour une confirmation, il est recommandé de préparer les sérums de contrôle en double.

C. Déposer 100 µL d'échantillon de lait dans un puits sélectionné recouvert d'antigène viral de BLV gp51.

   Pour une confirmation, il est recommandé de préparer les échantillons en double.

3. Agiter la plaque avec précaution. Fermer hermétiquement la plaque/barrette et incuber pendant 1 heure à 37°C.
4. Rincer les plaques/barrettes 3 fois avec le tampon PBS Tween: à chaque cycle de rinçage, remplir les puits et vidanger la plaque en tapant vigoureusement pour éliminer tout reste de fluide.
5. Déposer 100 µL de conjugué dans chaque puits et incuber pendant 1 heure à 37°C.
6. Répéter la procédure de lavage du point 4.
7. Déposer 100 µL de solution de substrat dans chaque puits. Incuber 10 minutes à température ambiante, entre 18-25°C. Commencer le décompte quand le premier puits est rempli.
8. Arrêter la réaction en ajoutant 50 µL de solution d'arrêt dans chaque puits et mélanger soigneusement. Placer la solution d'arrêt dans le même ordre que la solution de substrat au point 7.
9. Mesurer la densité optique (DO) des contrôles et des échantillons à 450 nm dans un photomètre à microplaques (utiliser l'air comme blanc).

   Mesurer la DO dans les 15 minutes suivant l'addition de solution d'arrêt pour éviter la fluctuation des valeurs de DO.

Calculs

Le calcul des résultats est effectué tel que décrit ci-dessous.

Calcul des valeurs de pourcentage de positivité (PP)

Calculer la valeur de densité optique moyenne (DO) pour chaque témoin contrôle.

Toutes les valeurs de DO concernant les échantillons tests ainsi que le contrôle négatif, sont calculées par rapport à la valeur de DO du contrôle positif comme suit:

$$PP = \frac{DO_{\text{échantillon/contrôle négatif}}}{DO_{\text{contrôle positif}}} X100$$

Interprétation des résultats

Critères de validité du test

Pour garantir la validité des tests, le double des valeurs de DO de chaque puits d'un contrôl positif ne doit pas s'écarter de plus de 25 % de la valeur moyenne du duplicata. De plus, les valeurs de contrôle doivent être comprises dans les limites suivantes:

DO Contrôle positif	**> 1,0**
PP Contrôle négatif	**< 15**

Si l'un de ces critères n'est pas satisfait, le test n'est pas validé. Pour les tests non validés, la technique peut être suspectée, et l'essai doit être répété.

Interprétation du resultat de l'échantillon test

Échantillon	PP	Interprétation
Sérum	< 20 ≥ 20	Négatif Positif
Lait	< 15 ≥ 15	Négatif Positif

Pour confirmer les résultats de l'échantillon test, chaque valeur PP distincte en double doit être interprétée de manière égale, à savoir positive et négative. En cas de divergence, il est recommandé d'analyser à nouveau l'échantillon.

Bibliographie

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2. Ferrer, J.,F. (1980) Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med. 24, 1-68.
3. Manet, G. *et al*. (1989). Natural mode of horizontal transmission of bovine leukemia virus (BLV): the potential role of tabanids (*Tabanus spp*.). Vet. Immunol. Immunopathol. 22, 255-263.
4. Evermann, J.F. *et al*. (1986). Transmission of bovine leucosis virus by blood inoculation. Am. J. Vet. Res. 47, 1885-1887.
5. Hoff-Joergensen, R. (1989). An international comparision of different laboratory tests for the diagnosis of bovine leucosis: suggestions for international standardization. Vet. Immunol. Immunopathol. 22, 293-297.

***DANGER: Solution d'arrêt (acide sulfurique).**
Peut être corrosif pour les métaux. Provoque une irritation cutanée. Provoque une sévère irritation des yeux. Conserver uniquement dans le récipient d'origine P280 Porter des gants de protection/des vêtements de protection/ un équipement de protection des yeux/du visage.
EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. Appeler immédiatement un CENTRE ANTIPOISON/ un médecin. Si l'irritation oculaire persiste: consulter un médecin .
EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment à l'eau. Enlever les vêtements contaminés et les laver avant réutilisation.
EN CAS D'IRRITATION CUTANEE: consulter un médecin. Absorber toute substance répandue pour éviter qu'elle attaque les matériaux environnants.

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Service à la clientèle
support@indical.com

Symbols / Symbolen / Símbolos / Symboles

REF	Article No. / Artikelnummer / Nº de artículo / Nº d'article
LOT	Serial (batch) No. / Ch.-B / Nº de lote / Nº de série (lot)
	Temperature limit / Lagerungstemperatur / Límite de temperatura / Limite de température
	Expiry date / Verwendbar bis / Fecha de caducidad / Date de péremption
	Number of samples / Anzahl der Proben / Nº de muestras / Nombre des échantillons
	See manual / Siehe Gebrauchsinformation / Ver el manual / Voir le manuel
	Manufacturer / Hersteller / Fabricante / Fabricant

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Manual version: HB-2620-001
September 2024

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

TÜV SÜD Product Service

Certificate

No. Q5 071067 0006 Rev. 03

Holder of Certificate: **Liofilchem S.r.l.**
Via Scozia
64026 Roseto degli Abruzzi (TE)
ITALY

Certification Mark:

TÜV SÜD EN ISO 13485 tuvsud.com/ps-cert

Scope of Certificate: **Design and development, production and sales of in vitro diagnostic culture media for bacteriology, mycology and parasitology, in vitro diagnostic controls/standards/calibrators for microbiology, in vitro diagnostic identification and susceptibility testing, and microbiology tests. Distribution of in vitro diagnostic reagents for clinical chemistry and immunochemistry.**

The Certification Body of TÜV SÜD Product Service GmbH certifies that the company mentioned above has established and is maintaining a quality management system, which meets the requirements of the listed standard(s). All applicable requirements of the Testing, Certification, Validation and Verification Regulations TÜV SÜD Group have to be complied with. For details and certificate validity see: www.tuvsud.com/ps-cert?q=cert:Q5 071067 0006 Rev. 03

Report No.: ITA200220002478

Valid from: 2024-12-19
Valid until: 2027-12-18

Date, 2024-09-16

Christoph Dicks
Head of Certification/Notified Body

TÜV SÜD Product Service GmbH • Certification Body • Ridlerstraße 65 • 80339 Munich • Germany

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TÜV SÜD Product Service

Certificate

No. Q5 071067 0006 Rev. 03

Applied Standard(s):

ISO 13485:2016
(EN ISO 13485:2016/AC:2018, EN ISO 13485:2016/A11:2021)
Medical devices - Quality management systems - Requirements for regulatory purposes

Facility(ies):

Liofilchem S.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE), ITALY

Production of in vitro diagnostic culture media for bacteriology, mycology and parasitology.

Liofilchem S.r.l.
Via Uruguay, 64026 Roseto degli Abruzzi (TE), ITALY

Design and development, production and sales of in vitro diagnostic culture media for bacteriology, mycology and parasitology, in vitro diagnostic controls/standards/calibrators for microbiology, in vitro diagnostic identification and susceptibility testing, and microbiology tests. Distribution of in vitro diagnostic reagents for clinical chemistry and immunochemistry.

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ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

Product Service

C E R T I F I C A T O

N°Q5 071067 0006 Rev. 03

Titolare del certificato: **Liofilchem S.r.l.**
Via Scozia
64026 Roseto degli Abruzzi (TE)
ITALIA

Marchio di certificazione:

Campo di applicazione:

Progettazione e sviluppo, produzione e vendita di dispositivi medico diagnostici in vitro: terreni di coltura per batteriologia, micologia e parassitologia, controlli/standard/calibratori per microbiologia, test di identificazione e di suscettibilità, e test di microbiologia. Distribuzione di reagenti diagnostici in vitro per chimica clinica e immunochimica.

L'Organismo di Certificazione TÜV SÜD Product Service GmbH certifica che la società sopramenzionata ha istituito e mantiene un sistema di gestione qualità conforme ai requisiti della(e) norma(e) elencata(e). Tutti i requisiti applicabili del Regolamento "Testing, Certification, Validation and Verification" del gruppo TÜV SÜD devono essere rispettati. Per dettagli e validità del certificato vedi: www.tuvsud.com/ps-cert?q=cert:Q5 071067 0006 Rev.

N° del rapporto: ITA200220002478

Valido da: 2024-12-19
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Data, 2024-09-16

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TÜV SÜD Product Service

C E R T I F I C A T O

N°Q5 071067 0006 Rev. 03

Norma(e) applicata(e): ISO 13485:2016
(EN ISO 13485:2016/AC:2018, EN ISO 13485:2016/A11:2021)
Dispositivi medicali - Sistemi di gestione qualità - Requisiti per scopi regolamentari

Stabilimento(i):

Liofilchem S.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE), ITALIA

Produzione di dispositivi medico diagnostici in vitro: terreni di coltura per batteriologia, micologia e parassitologia.

Liofilchem S.r.l.
Via Uruguay, 64026 Roseto degli Abruzzi (TE), ITALIA

Progettazione e sviluppo, produzione e vendita di dispositivi medico diagnostici in vitro: terreni di coltura per batteriologia, micologia e parassitologia, controlli/standard/calibratori per microbiologia, test di identificazione e di suscettibilità, e test di microbiologia. Distribuzione di reagenti diagnostici in vitro per chimica clinica e immunochimica.

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Swarm Agar

Medium for detection of H-phases of *Salmonella,* according to ISO 6579-3.

TYPICAL FORMULA	(g/l)
Meat Extract	5.0
Yeast Extract	1.0
Trypto-Casein Soya Broth	30.0
Glucose	1.0
Sodium Desoxycholate	0.35
Agar	7.0
Final pH 7.6 ± 0.2	

DESCRIPTION

Swarm Agar is a soft nutrient-rich medium used for determining the H-phases of *Salmonella* with the Sven Gard method.

The medium is compliant with the requirements in the ISO/TR 6579-3 for serotyping *Salmonella*.

PREPARATION

Melt the content of a tube in a water bath at 100°C (with the cap loosened) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the tube upside down. To the melted medium (10 ml), cooled at 47-50°C, add the specific anti-H Salmonella serums for phase inversion, corresponding to the previously-determined phase (see below for more details). Mix with circular movements, avoiding foam formation, and aseptically pour onto Petri dishes with a diameter of 55 mm.

PRINCIPLE

Salmonella nomenclature distinguishes between two species: *Salmonella bongori* and *Salmonella enterica*. The main specie, *Salmonella enterica*, is divided into six subspecies: I (*S. enterica* subsp. *enterica*), II (*S. enterica* subsp. *salamae*), IIIa (*S. enterica* subsp. *arizonae*), IIIb (S. *enterica* subsp. *diarizonae*), IV (*S. enterica* subsp. *houtenae*) and VI (*S. enterica* subsp. *indica*). Various serovars can be identified within each subspecie. They are characterised by their somatic (“O”), flagellar (“H”) and capsular (“Vi”) antigens which all together define the antigenic formula of a given serovar (serotype or ser).

The general procedure for serotyping an unknown *Salmonella* isolate is based on determining the antigenic formula with rapid slide agglutination test using specific sera raised primarily against the somatic (“O”) or flagellar (“H”) antigens; the serum raised against the “Vi” antigen is useful for the Dublin serotype. It is highly advisable to first identify the family, genus, species and subspecies based on morphological and biochemical traits before determining the serovar.

Salmonella generally have two types of H antigens (phase 1 or the specific phase and phase 2 or the non-specific phase). Some cultures are monophasic and may be directly H typed, whereas the second phase in a diphasic culture is determined with a phase inversion method like that of Sven Gard.

TECHNIQUE

Agglutination with H-antisera is performed after agglutination with the O-antisera. Once the first H antigen is identified, a phase inversion on the isolate is performed to force the organism to repress its dominant H phase and express the second phase. Phase 2 is determined by adding the phase 1 corresponding phase inversion antiserum to Swarm Agar which allows the bacteria that express the phase 2 H antigens to swarm. The procedure for detection of H antigens is described in more details below.

- Test the isolate with a polyvalent H antiserum.
- When the isolate is positive (agglutination) for a group, identify the H antigens by performing sequential tests of the monovalent H antisera for that group.
- When a phase 1 H antigen is determined, use the Sven Gard method as follow:
- Add 2 drops of specific phase inversion antiserum (corresponding to the flagellar phase already determined) to a Petri dish (55 mm) and mix with 10 ml Swarm Agar (maintained as soft agar at 47-50°C).
- Allow the medium to solidify on a levelled table.
- Inoculate the strain at a single point in the centre of the dish.
- Incubate the inoculated plate at 36 ± 2°C for 18-21 h.
- After incubation, take a culture at periphery of the invasion zone (swarm) of the Swarm Agar.
- Start testing again by using the H polyvalent antisera.
- If there is no agglutination, the serotype contains only one phase.
- If one of the groups shows agglutination, define the specific H phase by using the relevant H monovalent antisera.

INTERPRETATION OF RESULTS

Report the (full) name and, whenever possible, also the antigenic formula that has been determined by referring to the White–Kauffman–Le Minor scheme.

STORAGE

10-25°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. ISO/TR 6579-3:2014. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 3: Guidelines for serotyping of *Salmonella* spp.

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PRODUCT SPECIFICATIONS

NAME
Swarm Agar

PRESENTATION
Glass tubes containing semi-solid medium

STORAGE
10-25°C

PACKAGING

Ref.	Content	Packaging
26488	100 x 10 ml tubes	100 tubes in cardboard box

pH OF THE MEDIUM
7.6 ± 0.2

USE
Swarm Agar is used during serotyping of *Salmonella* to demonstrate the inapparent H antigen phase of diphasic isolates according to the Sven Gard method

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Amber, clear

SHELFLIFE
2 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Sterility control
   7 days at 22 ± 2°C, in aerobiosis
   7 days at 35 ± 2°C, in aerobiosis
3. Microbiological control
   Incubation Conditions: 36 ± 2°C / 18-21 h
   (Swarm Agar without serum)

Microorganism		Specification
Salmonella Typhimurium	ATCC® 14028	Good growth, intensive swarming

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instruction for use	

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Bile Aesculin Agar

Selective medium for the preliminary identification of group D streptococci.

TYPICAL FORMULA	(g/l)
Beef Exract	3.0
Meat Peptone	5.0
Esculin	1.0
Oxgall	40.0
Ferric Citrate	0.5
Agar	14.0
Final pH 6.4 ± 0.2 at 25°C	

DESCRIPTION

Bile Aesculin Agar is used for the selective isolation and differentiation of enterococci and streptococci (serology group D) from food and pharmaceutical products. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

PRINCIPLE

Beef extract and meat peptone provide amino acids, nitrogen, carbon, vitamins and minerals. Oxgall is ox bile purified and dehydrated. It contains a mix of biliary salts and is used in media for enterobacteria, as selective agent, because inhibit Gram-positive bacteria other than Group D streptococci. Esculin is a glucoside which is hydrolysed by Group D streptococci to form aesculetin and dextrose. Aesculetin combines with ferric citrate in the medium to form a dark brown or black complex which is indicative of a positive result. Agar is the solidifying agent.

PREPARATION

Suspend 63.5 g of powder in 1 liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C. Dispense in Petri dishes.

TECHNIQUE

Inoculate the medium by streaking directly the sample onto the agar surface.

Incubate at 36 ± 1°C for 18-24 hours in aerobic atmosphere.

INTERPRETATION OF RESULTS

Group D streptococci grow on this medium producing a dark brown color around the colonies.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. European Pharmacopoeia 9.0 (2017) - 2.6.12. Microbiological examination of non-sterile products: Microbial enumeration tests. 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. Swan, A. 1954.The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. 7:160.
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PRODUCT SPECIFICATIONS

NAME
Bile Aesculin Agar

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
620210	100 g	100 g of powder in plastic bottle
610210	500 g	500 g of powder in plastic bottle
6102105	5 kg	5 kg of powder in plastic bottle

pH OF THE MEDIUM
6.4 ± 0.2

USE
Bile Aesculin Agar is used for the selective isolation and differentiation of enterococci and streptococci (serology group D) from food and pharmaceutical products

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: greenish, light to medium beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: greenish to medium amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation Conditions: 24 h/ 36 ± 1°C

Microorganism		Growth	Characteristics
Enterococcus faecalis	ATCC 19433	Good	Blackening of the medium
Streptococcus pyogenes	ATCC 19615	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	Consult instructions for use	Manufacturer	Use by
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Keep away from sunlight

LIOFILCHEM® S.r.l.
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Instructions For Use

ENGLISH

Slanetz Bartley Agar (m-Enterococcus)
Slanetz Bartley Agar + TTC
Slanetz Bartley Agar Base

Selective medium for detection and enumeration of intestinal enterococci, according to ISO 7899-2.

INTENDED PURPOSE

Selective medium for the isolation and enumeration of enterococci in water and other samples. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

DESCRIPTION

Slanetz Bartley Agar is recommended by ISO 7899-2 for detection and enumeration of intestinal enterococci in water by membrane filtration. This medium can be also used by direct plating for the examination of foodstuffs or other materials. The presence of sodium azide inhibits the development of all contaminating microorganisms, whilst triphenyltrazolium chloride (TTC) is reduced to formazan by enterococci, which grow as red to maroon colonies.

TYPICAL FORMULA* (Per Litre of Purified Water)

Tryptose	20.0 g
Glucose	2.0 g
Yeast Extract	10.0 g
Dipotassium Hydrogen Phosphate (K_2HPO_4)	4.0 g
Sodium Azide (NaN_3)	0.4 g
2,3,5-triphenyltetrazolium chloride (TTC)	0.1 g
Agar	13.0 g
Final pH 7.2 ± 0.1 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

Note: Slanetz Bartley Agar is available both as complete medium and agar base to be used with TTC 1% supplement (see the ORDER INFORMATON section).

METHOD PRINCIPLE

Tryptose provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of group B. Glucose is the fermentable carbohydrate providing carbon and energy. Dipotassium hydrogen phosphate is a buffer. Sodium azide inhibits Gram-negative bacteria and staphylococci. TTC is a redox indicator of bacterial growth, which is colorless in the oxidized form and is reduced to the insoluble red triphenyl formazan. Agar is the solidifying agent.

PREPARATION

Dehydrated medium with TTC

Suspend 44.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil until completely dissolved. DO NOT AUTOCLAVE. Dispense an appropriate volume into plates, e.g. pour 20 ml of medium into Petri dishes of 90 mm.

Dehydrated medium without TTC

Suspend 44.4 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil until completely dissolved. DO NOT AUTOCLAVE. Cool to 45-50°C. Add 10 ml of TTC 1% supplement before distributing into Petri dishes.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Test tubes, inoculating loops, incubator, quality control organisms.

TEST PROCEDURE

Ensure there is no visible moisture on the plates before use.

For the examination of water, filter 100 ml of the sample trough a filter membrane (0.45 µm pore diameter), and transfer this onto the surface of the medium.

For other samples, dilute as necessary and spread 0.5 ml over the agar surface.

Incubate aerobically at 36 ± 2°C for 40-48 hours.

For more details, consult appropriate guidance.

INTERPRETING RESULTS

Typical colonies show a red, maroon or pink color, either in the centre or throughout the colony.

Note: TTC reduction to formazan is not exclusive to enterococci, which may be confirmed by demonstrating aesculin hydrolysis reaction.

Following ISO 7899-2, confirm by transferring the membrane and the colonies onto a plate of Bile Aesculin Azide Agar pre-warmed to 44°C. Incubate at 44 ± 0.5°C for 2 hours. Count all typical colonies showing a brown to black color in the surrounding medium as intestinal enterococci.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed.

Store prepared plates at 10-25°C away from direct light in their original pack until just prior to use. Avoid quick temperature shifts of plated medium to prevent condensation.

Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Ready-to-use plates: 6 months.
Supplement: 1 year.

QUALITY CONTROL

Appearance of TTC 1% supplement: clear, colourless.

Appearance of dehydrated medium: Free-flowing, homogeneous, light beige.

Appearance of prepared medium: Light amber, slightly opalescent.

Expected Cultural Response:

Control strain	Inoculum	Incubation	Criteria	Specification
Enterococcus faecalis WDCM 00087 (ATCC® 29212; NCTC 12697)	50-100 CFU	40-48 h / 36 ± 2°C	Good growth ($P_R \geq 0.5$)	Red-maroon-pink colonies
Enterococcus faecalis WDCM 00009 (ATCC® 19433; NCTC 775)				Red-maroon-pink colonies
Enterococcus faecium WDCM 00177 (ATCC® 6057)				Inhibition
Escherichia coli WDCM 00013 (ATCC® 25922; NCTC 12241)	10^4-10^6 CFU		Inhibition	---
Staphylococcus aureus WDCM 00034 (ATCC® 25923)			Inhibition	---

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of Slanetz Bartley Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the

principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

ORDER INFORMATION

Product	Format	Packaging	Ref.
Slanetz Bartley Agar (m-Enterococcus A.) [a]	Plate 60 mm	20 plates, in blister of 2 pieces (double wrapped)	163462
Slanetz Bartley Agar (m-Enterococcus) [a]	Plate 90 mm	20 (2 x 10) plates	11058
Slanetz Bartley Agar + TTC [a]	Dehydrated medium	100 g	620147
		500 g	610147
		5 kg	6101475
Slanetz Bartley Agar Base [b]	Dehydrated medium	100 g	620134
		500 g	610134
TTC 1% supplement	Bottle	5 x 10 ml	80300
	Bottle	10 x 10 ml	80430

Footnotes:

a) Complete medium (includes TTC)
b) Medium without TTC, requiring supplementation with TTC 1% supplement

Revision History

Revision	Release Date	Change Summary
0	2024-07-02	Updated layout and content, version reset to revision 0

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

References

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media.
2. ISO 7899-2:2000. Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method.
3. Burkwal, M.K., and P.A. Hartman. 1964. App. Microbiol. 12,18.
4. Slanetz, L.W., and C.H. Bartley 1957. J. Bact., 74,591.

Table of Symbols

LOT	Batch code
REF	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

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