

# Avian Pneumovirus (APV-Ab)

## SVANOVIR®

ELISA test for the detection of APV antibodies in serum and egg yolk

### General Information

Avian Pneumovirus (APV), previously known as *Turkey Rhinotracheitis (TRT) virus*, causes acute, highly contagious upper respiratory tract disease in turkeys and chicken. The disease in turkeys have been reported from several European countries, South Africa and Israel since 1985. Typical clinical signs of the disease include snicking, nasal discharge, conjunctivitis, swollen sinuses and a marked drop in egg production. The virus can also infect chicken without developing any clinical signs.

However, swollen head syndrome of chicken has been reported in APV-infected chicken flocks. Outbreak of APV, associated with adventitious infections or poor management can lead to heavy economical loss. The infection does not usually give lifelong protective immunity and yet there is very little described correlation between the level of antibodies and protection against the disease. Local immunity may play a bigger role in the protection against disease than the circulating antibodies.

### Principle

The SVANOVIR® Avian Pneumovirus-Ab ELISA Kit is designed to detect APV specific antibodies in serum samples and egg yolk. The kit procedure is based on the Blocking Enzyme Linked Immunosorbent Assay (Blocking-ELISA). In this procedure, samples are exposed to noninfectious APV antigen coated wells in microtitre plates. If antibodies directed to APV are present in the test sample, they will bind

virus antigens in the well and block these antigen sites. If antibodies directed to APV are absent in the sample these sites will remain free. When horseradish peroxidase (HRP) conjugated monoclonal antibodies directed to APV are added, they will bind to these free sites on the virus. After addition of the substrate a blue colour develops.

A negative result is indicated by a strong colour change. 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. OD readings from the samples are compared to those of the positive and negative control sera.

### Contents

- APV antigen coated microtitre plates (sealed and stored dry)
- Lyophilized HRP Conjugate (Horseradish Peroxidase conjugated anti-APV-monoclonal antibodies)
- PBS-Tween Solution 20 x concentrate
- Substrate Solution – (tetramethylbenzidine in substrate buffer containing  $H_2O_2$ ) – **STORE IN THE DARK!**
- Stop Solution – Contains sulphuric acid – **CORROSIVE!**
- A. Positive Control Solution – Serum diluted in PBS-Tween containing 0,05% merthiolate

B. Negative Control Serum – 0,05% merthiolate

stored at -20°C for 4 weeks and thawed and refrozen up to 3 times.

### Material needed but not provided

1. Precision pipets (range from 5 to 200 µl)
2. Disposable pipet tips
3. Distilled water
4. Wash bottle
5. Container: 1 to 2 litres for Washing Buffer
6. Microplate photometer, 450 nm filter

### Specimen information

50 µl of undiluted blood serum or plasma is required for each sample well. Fresh, refrigerated, previously frozen serum or plasma can be used. If egg yolk is used for testing, 20 µl of undiluted fresh egg yolk is required for each sample well.

### 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 PBST 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.

**Anti-APV Conjugate:** Reconstitute the lyophilized HRP Conjugate with 11.5 ml PBS-Tween Buffer. Add the buffer carefully to the bottle. Leave the solution one minute and mix thoroughly. Prepare immediately before use. The remaining reconstituted conjugate can be

### Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at +2 to +8°C (35 to 45°F)
3. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction booklets 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 expire.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipet tip for each sample.
10. Do not pipet by mouth.
11. Include positive Control Solution and negative Control Serum on each plate or test strip series.
12. Use only distilled water for preparation of reagents.
13. The Stop Solution contains sulphuric acid, which is corrosive.
14. All unused biological materials should be disposed according to the local, regional and national regulations.

### Recommendations!

Reconstituted conjugate may not be stored in refrigerator.

The volume of the reagents is sufficient for at least 10 separate test occasions.

Strips with broken seal can be stored at +2 to +8°C for up to 4 weeks.

## Procedure

1. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use. Label each strip with a number.
2. Add 100 µl of Positive Control Solution (A) and 100 µl of Negative Control Serum (B) to selected wells. For confirmation purposes it is recommended to run the control sera in duplicates.
3. Add 50 µl of PBS-Tween Buffer and 50 µl undiluted serum sample to each of the selected wells and mix thoroughly.  
If egg yolk is tested, mix egg yolk thoroughly with PBS-Tween Buffer (dilution 1 in 5) before adding the mixture to the microtitre wells (100 µl/well).
4. Seal the plate and incubate for 30 minutes at room temperature 18 to 25°C, (64 to 77°F).
5. 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.
6. Add 100 µl of HRP Conjugate to each well.
7. Seal the plate and incubate for 30 minutes at room temperature 18 to 25°C, (64 to 77°F).
8. Repeat step #5.
9. Add 100 µl Substrate Solution to each well. Incubate for 10 minutes at room temperature 18 to 25°C, (64 to 77°F). Begin timing when the first well is filled.
10. Stop the reaction by adding 50 µl Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #9.
11. 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

1. Calculate the mean OD-values for each of the controls and samples.
2. Calculate the percent inhibition (PI) values for positive control as well as samples, using the following formula:

$$PI = \frac{(OD_{Neg\ ctrl} - OD_{Sample/Pos\ ctrl}) \times 100}{OD_{Neg\ ctrl}}$$

## Interpretation of results

### Criteria for test validity

To ensure assay validity, the Negative Control Serum should have an optical density (OD) value greater than 0.600. The Positive Control Solution should have a PI greater than 40. For invalid tests, technique may be suspect and the assay should be repeated.

### Interpretation

PI > 40	positive
PI 30–40	doubtful
PI < 30	negative

Samples with a PI between 30–40 are considered doubtful and should be retested. If the results are still doubtful the flock should be sampled and tested again within 10–14 days.

This manual covers the following APV-Ab ELISA kits: article number 10-1100-02

## **Manufacturer**

### **Svanova Biotech AB**

Uppsala Science Park

SE-751 83 Uppsala, Sweden

Phone +46 18 65 49 00 Fax +46 18 65 49 99

info@svanova.com www.svanova.com

## **Customer Service**

Phone +46 18 65 49 15 Fax +46 18 65 49 99

customer.service@svanova.com

Manualnumber: 19-1100-00/05