



NAME AND INTENDED USE

Autolex® Anti-PRV Screen, manufactured by Calbiotech Veterinary Diagnostics, is a semi-automated latex agglutination immunoassay for the detection of antibodies to pseudorabies virus in swine serum.

INTRODUCTION

Pseudorabies virus infection, which causes Aujeszky's disease, occurs naturally in many species. In adult swine, the disease symptoms usually are mild and the mortality is low. However, in other animals the disease is highly fatal. Swine are the primary hosts of pseudorabies virus and it is transmitted from the infected pigs to others in the herd. In pigs that have recovered from Aujeszky's disease, the pseudorabies virus remains in a latent state where it resides quiescently in the trigeminal ganglion (1,2). Upon stimulation, the virus may reactivate and produce an active infection. Although clinical illness may not be seen in swine after recrudescence, the virus may be shed by these animals (1,3,4). Therefore, any animal that has contracted a primary infection by pseudorabies virus must be considered a potential source for transmission of infectious virus.

Control of pseudorabies virus depends on elimination of the reservoir of infection. This requires the identification of any swine that have experienced an infection, since these animals should be considered potentially infectious due to recrudescence. Any previous or current infection with pseudorabies virus results in the

presence of specific antibodies in the serum and these are a good marker of infection.

The Autolex Anti-PRV Screen is a semi-automated assay designed as an adaptation of the manually-formatted latex agglutination test (LAT) also manufactured by Calbiotech Veterinary Diagnostics. While utilizing the same latex agglutination principles as LAT, the Autolex test is performed in specially selected 96-well microplates and read by a spectrophotometer and proprietary software. This offers the user faster throughput, objective analysis and hard copy results while retaining the established sensitivity and specificity of LAT (5).

PRINCIPLES AND DESCRIPTION

A. Latex The Autolex Anti-PRV Screen utilizes a latex reagent which consists of polystyrene microspheres sensitized with pseudorabies (PRV) virus components. When this reagent is mixed with diluted swine serum in specially selected flat-bottomed microwells, antibodies to PRV will agglutinate the latex. Sera that do not contain antibodies to PRV will not agglutinate the latex particles, and the latex will remain suspended in a uniform manner.

B. Reader The Bio-Tek Ceres 900 and ELX 808 Scanning Autoreader utilize fiber optics to read flat-bottomed 96-well microplates. The reader quickly scans each well and records the light transmission at multiple points. Proprietary software records the data and formats the outcome for hard copy results.

C. Preparation There is no preparation of any reagents in the Autolex assay. Reagents must be allowed to warm to room temperature (21 to 25°C) before use.

D. Assay There are two readings for each microplate. The initial reading – performed after the addition of all reagents, sera and controls – is a blank for each well to establish the initial value. After mixing the reagents and sera by rotation, a final reading is taken. In a sample containing PRV antibodies, the latex aggregates allow additional light to pass through the well. In a negative sample, the latex remains evenly dispersed, so the light transmission shows little change.

E. Outcome The Autolex software subtracts the initial value from the final reading to obtain the change in percent transmission of light. Positive and negative controls are included to calibrate each plate and to calculate the Autolex Index for the samples. Index values greater than 8.0 are positive for pseudorabies virus antibodies, less than 4.5 are negative, and values from 8.0 to 4.5 are indicated to be retested using the manual LAT assay (see Results).

MATERIALS PROVIDED

Product #9022

Materials provided for 960 determinations include:

Autolex PRV Latex Reagent	Ten (10) 2.8 mL bottles
Autolex PRV Sample Dilution Buffer	Two (2) 125 mL bottles
Autolex PRV Positive Control Serum	One (1) 2.0 mL bottle
Autolex PRV Negative Control Serum	One (1) 2.0 mL bottle
Costar Cell Culture Cluster Dish	Ten (10) microplates

MATERIALS REQUIRED BUT NOT PROVIDED

Ceres 900 or ELX 808 Scanning Autoreader, Bio-Tek Instruments, Inc.

VAI Rotator, Model 2342

Pipettors for delivery of 25, 50 and 150 microliters

Disposable pipette tips

Dilution cups or tubes

Reagent reservoirs

GENERAL PRECAUTIONS

Use good laboratory techniques and follow the procedure.

Reagents must be stored at 2 to 7°C.

For veterinary use only.

Do not expose the reagents to temperatures below 2°C or above 32°C.

Use each microwell once and dispose of microtiter plate properly.

All disposables should be used only once and discarded.

WARNINGS

1. This test is formatted to use 25 $\mu\text{l} \pm 3 \mu\text{l}$ of the Latex Reagent. This will produce initial transmittance values for control sera of 9 to 17, with an average range of 11 to 14. The initial transmittance must remain within 9 to 17 to achieve proper test sensitivity. If consistently high or low values are obtained, the accuracy of the pipettor should be verified and calibrated.
2. An error flag of an (L) or (H) is printed beside the initial transmittance values for samples that fall outside of acceptable values. This could result from an incorrect delivery volume of the Latex Reagent or from serum samples containing excessive particulate matter. These samples should be retested.
3. If the test controls fall outside acceptable values as judged by the Autolex software, a message "Calibrator replicates out of

specification” is printed at the top of the report. The test results are invalid and the plate should be repeated.

4. Use only Costar Cell Culture Dish, flat-bottomed microplates (Costar product #3596) as provided through Calbiotech Veterinary Diagnostics. Use of other plates will provide erroneous results.
5. Unused Latex Reagent must be returned immediately to vials and capped. Latex reagent must not remain in the reagent reservoir for longer than 30 minutes during test procedure.
6. The Latex Reagent and Sample Dilution Buffer contained in the manually formatted Pseudorabies Virus Antibody Test Kit-Latex Agglutination (Product #'s 9017 and 9018) are not compatible with the Autolex System and cannot be substituted.
7. Positive and Negative Control Sera are matched to each lot of Latex Reagent and are not interchangeable. Do not use controls from different lot numbers.
8. Diluted Negative Control Sera must be placed in wells A1, A2 and A3. Diluted Positive Control Sera must be placed in wells A4, A5 and A6.
9. The Autolex reagents contain small amounts of sodium azide which may react with lead and copper plumbing to form potentially explosive compounds. Flush with large amounts of water upon disposal.

SPECIMEN INFORMATION

Serum is the specimen of choice. Plasma has not been evaluated with the Autolex procedure. Samples may be stored at 2 to 7°C for up to 48 hours or may be stored frozen at minus 10°C or below for extended periods.

Hemolysis does not affect test results. Sera with heavy microbiological contamination should not be assayed.

PREPARATION OF SAMPLES

Dilute all samples and controls four-fold (1:4) with Sample Dilution Buffer and mix. (see Procedure for example).

AUTOLEX® PROCEDURE

1. Remove reagents from refrigerator and allow to warm to room temperature (21 to 25°C).
2. Arrange samples in an 8x12 rack, using the first six tubes of the rack (A1, A2, A3, A4, A5, A6) for controls.
3. Transfer Sample Dilution Buffer into a clean reagent reservoir. Using a multichannel pipettor, dispense 150 µl of buffer into dilution cups or tubes.
4. Place 50 µl of Negative Control Serum into dilution cups A1, A2 and A3. Transfer 50 µl of Positive Control Serum into

dilution cups A4, A5 and A6. Place 50 µl of test serum samples into all subsequent diluent cups.

5. Vortex a bottle of Latex Reagent for 10 seconds and pour into a new reagent reservoir.
6. Using a multichannel pipettor, immediately transfer 25 µl of Latex Reagent into each well of a Costar microplate (see Warnings). Avoid the formation of bubbles.
7. Using a multichannel pipettor and new tips for each row of samples, gently mix diluted sera by drawing up and down five times. Then, transfer 25 µl of each sample into the microtiter plate wells. Avoid the formation of bubbles.
8. Perform the initial reading of the test by inserting the plate into a Ceres 900 or ELX 808 Scanning Autoreader. (see Autolex Operator’s Manual for detailed instructions)
9. After performing the initial reading, immediately mix the reagents by rotating the plate on a VAI Rotator, Model 2342 for thirty (30) minutes.
10. Return the plate to the spectrophotometer for a final reading. Rotation time must not exceed 33 minutes or a timing error message will appear on the results sheet.
11. Results will be printed out automatically and stored for future retrieval.

RETURN REAGENTS TO REFRIGERATOR IMMEDIATELY AFTER USE.

RESULTS

The Autolex software subtracts the initial reading from the final reading (after 30 minute mixing). The change in percent light transmission is recorded for each well. Each plate is calibrated using the controls located in the first six wells to produce an Autolex Index for each sample (see Autolex Operators manual).

An Autolex Index of less than 4.5 is negative for PRV antibody. An Autolex Index of greater than 8.0 is recorded as positive for PRV antibody. Tests falling between these values should be retested with the manually - formatted Pseudorabies Virus Antibody Test Kit-Latex Agglutination (product #9017 or #9018).

INTERPRETATION OF RESULTS

The Autolex software prints the assay results as shown in this example:

Case No.	Tube No.	Well No.	%T Initial	%T Final	Autolex Index	Result
01	1	A7	15.75	33.72	18.07	+
	2	A8	14.76	16.68	1.69	-
	3	A9	15.35	20.34	5.74	R
Index			PRV Result			
greater than 8.0			(+) Positive			
4.50 to 8.00			(R) Retest			
less than 4.5			(-) Negative			

Tube 1: Positive. The initial value is 15.75. The second (final) reading is 33.72. The software prints the difference between the

two values representing the converted change in light transmission after mixing (rotating). Therefore, Well A7 has an Autolex Index of 18.07 and should be reported as Positive for PRV antibodies.

Tube 2: Negative. An Autolex Index of 1.69.

Tube 3: Retest. An Autolex Index of 5.74. Specimen should be retested using the manually - formatted LAT.

TECHNICAL SERVICES

If you have any questions regarding the use of this test system, please call the manufacturer, **Calbiotech Veterinary Diagnostics** technical services at (619) 660-6162.

REFERENCES

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U.S. Patent No. 4,804,624 and 4,695,537

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