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VRL Laboratories

TEST KIT

PA1 DIA

(BV, STLV, SIV, SRV & Measles IgG Antibody)

FOR RESEARCH USE ONLY
NOT SUITABLE FOR HUMAN USE
READ ENTIRE INSERT PRIOR TO TESTING

Mar 2016

INTRODUCTION:

Simian Viruses Dot Immuno Assay (DIA) Panel A1 (catalog # D-085011 -100) is designed for the detection of 5 simian virus (B virus, STLV-1, SIV, SRV and Measles) IgG antibodies in sera that are mandatory to be tested in SPF non-human primates. This kit is based on the dot-blot immuno reaction, using the nitrocellulose membrane as the solid phase. The above 5 viral antigens and 2 cell culture controls are dotted on the membrane in 3 column x 9 row pattern (27 test sites total). Each test site is dotted with Vero cells, B virus, STLV, SIV, Measles, SRV and Raji cells . Testing sera absorbent filter paper strips are placed on the designated test site. Any viral antibodies present in the specimen will specifically react with their corresponding antigens on the DIA membrane. These antigen-antibody complexes are then reacted with the secondary antibody enzyme conjugate and are detected by adding a chromogenic substrate to develop into visually readable blue color. This is a qualitative detection of viral antibody to viral antigen in monkey serum. It can also be used for quantitative detection if the image of DIA membrane is captured by a scanner or digital camera.

TEST RESULTS AND INTERPRETATION:

To read the test results on the DIA membrane, use the Positive Controls and COLOR CHART as a guide for interpreting specimen test sites.

Positive (P): If cell culture control dot is colorless or weak blue, and the staining intensity of viral antigen dot is greater than or equal to weak positive control dot (+ ~ +++ reading), report the result as positive.

Indeterminate (I): If the staining intensity of viral antigen dot is greater than cell culture control dot (SRV v.s. Raji; BV/STLV/SIV/Measles v.s. Vero), but is less than weak positive control (-/+ reading), report the result as indeterminate. If viral antigen dot and its cell culture control are both darker than weak positive control (++ ~ +++ reading) and of equal intensity, report it as indeterminate.

Negative (N): If viral antigen dot is colorless, or the staining intensity is very weak and close to the intensity of its cell culture control, report the result as negative.

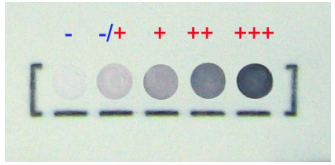
Any B virus and STLV-1 indeterminate specimen, it is suggested to re-draw new specimen two weeks later for retesting. If still indeterminate, it is recommended that both specimens are sent to VRL laboratories for multiple confirmations by DIA, ELISA and Western Blotting. For those SIV and SRV positive or indeterminate specimens, anticoagulated whole blood is suggested to be sent to VRL laboratories for further confirmation by Western Blotting or PCR. For animals tested Measles negative or indeterminate, we recommend to vaccinate them.

QUALITY CONTROL:

The provided positive and negative controls are representative of expected results and are to be included when testing is performed. These sera have been repetitively tested over time. The STLV positive control represents BV positive (++reading) and and STLV positive (+ reading). The BV positive control represents weak SRV positive (-/+ reading) and BV positive (+ reading), served as “Cut-of-Value”. The negative control represents a negative readings (absence of staining).

IF THE BV AND STLV POSITIVE CONTROLS ARE NOT BLUE (BV dot + and STLV+ reading, respectively), THE TEST IS INVALID.

READING CHART：



f. Place positive and negative serum control filter paper strips onto the last three test sites of the membrane. In the placement of test filter paper strips, make certain that each test strip does not touch adjacent strips to cause cross contamination.
g. Record the order of each specimen placement test site in detail.
h. After placing serum-saturated filter paper strips onto the membrane, place the cover on the test dish and incubate at 37°C for 30 minutes.

4. Washing

a. After incubation, remove and discard all serum-saturated filter paper strips by washing the membrane with pre-cold 1x wash solution .
b. Remove the filter paper pad and wash DIA membrane 3 times with 1x wash solution for 5 minutes each wash. Agitate the petri dish by placing it on a shaker during each wash.

5. Secondary antibody conjugate (AP conjugate)

a. Dilute provided secondary antibody conjugate 1:1000 in 1x wash solution (add 15 µl of AP conjugate stock into 15 ml of 1x wash solution and mix well).
b. Pour 15ml diluted conjugate onto the membrane and agitate gently to distribute evenly. Incubate at RT for 30 minutes.

6. Washing

a. Pour off the conjugate solution and wash the membrane 3 times with 1x wash solution for 5 minutes per each wash. Agitate on a shaker at medium speed during the wash.
b. Decant final wash as much as possible.

7. Color development

a. For each membrane, add 15 ml chromogen solution onto the membrane.
b. Gently agitate the dish until the positive controls develop to the optimal staining intensity, i.e. STLV dot of the positive control (STLV+) appears a 1+ reading and BV dot of the positive control (BV+) appears a 1+ reading (see below COLOR CHART) and its background is low (this takes about 2 to 5 minutes).
c. Terminate the reaction by washing the membrane in running tap water for a few seconds and allow the membrane to air dry.
d. Read the result when the membrane is dry.

MATERIAL PROVIDED:

No	Component	Quantity (container)	Storage	Description	Usage/ Membrane
1	Sample diluent	5 gx2 (foil bag)	4°C (keep dry)	Prepare 5% sample diluent in 1xWash Solution	1.5 g (30 ml 1xWash Solution)
2	Concentrated Wash Buffer	50ml (white bottle)	4°C	Dilute with dH_2O to make 1xWash Solution	
3	Chromogen	60 ml (amber bottle)	4°C	Bring up to room temperature before use	15ml
4	AP conjugate	60 µl (black top microtube)	4°C	Dilute 1:1000 by 1xWash Solution before use	15 µl (15ml 1xWash Solution)
5	Positive Control (STLV+)	200 µl (purple top microtube)	4°C	Dilute 1:5 by sample dilutant before use	
6	Positive Control (BV+)	200 µl (red top microtube)	4°C	Dilute 1:5 by sample dilutant before use	
7	Negative Control	200 µl (green top microtube)	4°C	Dilute 1:5 by sample dilutant before use	
8	PA1 DIA membrane	4 pieces (10x10 cm petri dish)	4°C (keep dry)	Block membrane before use	
9	Filter paper strips	120 strips (self-sealing bag)	RT	Use for adsorbing test serum	
10	Filter paper pad	5 pads (self-sealing bag)	RT	Put a pad under each DIA membrane to keep it moist during the primary Ag-Ab reaction.	

MATERIALS REQUIRED (NOT PROVIDED):

1. Forceps(a tip forceps and a blunt-ended forceps);
2. Distilled water (dH_2O) or deionized water;
3. 12x55 mm test tube for sera dilution;
4. 50 ml centrifugation tube, 250 ml and 1000 ml autoclavable container (flask);
5. 37°C incubator or water bath;
6. Shaker;
7. Vortex mixer;
8. 20 µl, 100 µl, and 1000 µl pipettes and tips;
9. Repeater Pipette and 5 ml (or 10 ml) pipette tips;
10. 50ml Eppendorf varispenser;
11. Electronic balance (readability 0.1 g).

SPECIMEN COLLECTION AND STORAGE:

1. Use a blood collection tube (without any anticoagulant) to collect approximately 1.5 ml venous blood. Be careful to avoid hemolysis.
2. Wait 30 minutes until blood clot is formed, then centrifuge at 3000 rpm for 10-15 minutes.
3. Aspirate serum from the blood collection tube and transfer to a 1.5-2.5 ml O-ringed screw-capped microtube. **NOTE: You must change pipette tips between specimens to avoid cross contamination.**
4. Specimens may be temporarily stored at 4°C; if storage period is to exceed 5 days, then the specimen should be frozen at -25°C.

REAGENT PREPARATION:

1. Wash Solution Pour 50ml concentrated wash buffer into a one-liter reagent bottle and add dH_2O up to 1000 ml to make 1x wash solution. Keep at 4°C and it can be stored for 2 months.
2. Sample Diluent For each DIA membrane, take 1.5 g of sample diluent powder (foil bag) into 30 ml of 1x wash solution to make 5% sample diluent. Use it immediately or store at 4°C up to 2 days.
3. AP Conjugate Solution Dilute AP Conjugate (black top microtube) 1:1000 in 1x wash solution.

PROCEDURE:

1. Sera dilution

- **a.** Label each serum specimen and its corresponding 12x55 mm test tube.
- **b.** Place test tubes on the test tube rack, and then add 400 µl of 5% sample diluent into each test tube.
- **c.** Add 100 µl of serum specimen into its corresponding tube.
- **d.** For controls, add 50µl controls into each tube, then and 200 µl of 5% sample diluent to the positive and negative controls tube individually, to make 1:5 dilution.

Note: Change tips between specimens to avoid cross contamination.

2. Blocking membrane

- **a.** Wear gloves to handle DIA membranes.
- **b.** Label each membrane and place them in a 10x10 cm plastic petri dish (1 piece/dish). Add 15 ml of 5% sample diluent and agitate on the shaker to block the membrane for 30 min at room temperature.
- **c.** After blocking, wash membrane three times with 1x wash solution for one minute each wash.
- **d.** Place the membrane on a filter paper pad and put in a 10x10 cm petri dish.
- **e.** Saturate the membrane and filter paper pad with 1x wash solution.
- **f.** Drain the excess 1x wash solution off the membrane without excess liquid on its surface.

3. Primary viral Ag-sera Ab reaction

- a. Mix diluted serum tube by vortexing.
- b. Use a fine tip forceps to hold one end of a filter paper strip, dip the strip in the diluted serum briefly.
- c. Drain excess serum off the strip by passing over the lip of the tube 1 to 2 times and place the strip directly on the designated test site on the DIA membrane. Each membrane has 3 columns and 9 rows, total 27 test sites for testing 24 specimens, one STLV positive (STLV+), one BV positive (BV+) and one negative control (NC).
- d. Dry forceps to paper toweling and then repeat placement of the next test filter paper strip. (**Note: Touch toweling in a different spot each time. This toweling is considered to be contaminated where the forceps have touched.**)
- e. Repeat placement of test filter paper strips for each individual's serum specimen on subsequent sites from top to bottom in the first left column and then go across to the second (middle) column, followed by the third (right) column.