

I. INTRODUCTION

ViraCHEK®/EIA uses a highly purified recombinant antigen to quickly identify antibodies to EIA in infected equines without causing the non-specific reactions commonly found in cultured antigen ELISA tests. ViraCHEK®/EIA has been optimized to use serum specimens. Results can be obtained in less than 20 minutes. The correlation between ViraCHEK®/EIA and LAB-EZ™/EIA is greater than 99%.

II. TEST PRINCIPLES

The plastic wells are coated with EIA recombinant antigen. The same EIA recombinant is labeled with the enzyme Horseradish peroxidase (HRP). The serum sample is incubated simultaneously with the coated wells and enzyme-labeled EIA antigen. Antibodies to EIA, if present in the equine sample, are bound to the well and enzyme-labeled EIA antigen at the same time. The free enzyme-labeled EIA antigen is washed away and a chromogenic substrate is added. The development of a dark blue color indicates the presence of antibody to EIA. In the absence of EIA antibody, little or no color change will be observed.

ViraCHEK®/EIA is highly specific, sensitive and simple to perform. Test results can be obtained in 20 minutes. The diagnostic kit contains a POSITIVE CONTROL and a NEGATIVE CONTROL which MUST be included each time

VI. CONTENTS OF ViraCHEK®/EIA TEST KIT

EIA Antigen	48 Test	96 Test
Coated Wells	4 X 12	8 X 12
Bottle A - EIA antigen - HRP conjugate (Blue Cap)	2.5 ml	5.0 ml
Bottle B - EIA Positive Control (Red Cap)	1.0 ml	1.6 ml
Bottle C - EIA Negative Control (Grey Cap)	1.0 ml	1.6 ml
Bottle D - Chromogen (Green Cap)	2.5 ml	7.5 ml
Bottle E - Substrate Buffer (White Cap)	2.5 ml	7.5 ml
Bottle F - 10X Wash Concentrate	100 ml	100 ml
Microwell holder		

Additional material required but not provided:

- 50 µl pipet; disposable pipet tips
- Deionized or distilled water
- Squirt Bottles (2); Timer
- Optional: Microwell plate reader

VII. PRECAUTIONS

1. Allow kit to come to room temperature (21°-25° C; 70°-78° F) prior to use.
2. Use separate pipet tip for each sample.
3. Do not expose kit to direct sunlight.
4. Do not use expired reagents or mix from different kit lots.

the assay is performed. Visual comparison of the color of the sample to the POSITIVE CONTROL will allow accurate detection of the presence of EIA antibody in the sample. If desired, test results may be determined by use of a microwell plate reader.

III. SAMPLE INFORMATION

50 µl of serum is required. Use only equine samples for test specimens. Samples may be stored at 2°-7°C for up to seven days. If longer storage is desired, samples may be stored at -20°C. Severely hemolyzed or lipemic serum may produce background color. When in doubt, obtain a better quality sample.

IV. PREPARATION OF WASH SOLUTION

Allow 10X Wash Concentrate to come to room temperature. Mix gently by inversion. Dilute wash concentrate 10-fold (1 part concentrate to 9 parts distilled water) in a wash bottle.

V. RESULTS

A. CONTROLS - For a valid test, the Positive Control must produce a distinct blue color and the Negative Control must produce no color. If color does not develop in the Positive Control or if there is any color development in the Negative Control well, results are invalid and the test should be repeated.

5. Follow instructions exactly. Improper washing or contamination of reagents may produce nonspecific color development.
6. For Veterinary Use Only.
7. Sale and use restricted to laboratories approved by State and Federal (U.S.D.A.) animal health officials.

VIII. STORAGE AND STABILITY

Store the test kit and unused diluted wash solution at 2°-7°C (36°-45°F). Do not freeze. Reagents are stable until expiration date provided they have been stored properly.

FOR TECHNICAL ASSISTANCE: 1-800-228-4305

REFERENCES

1. Merck Veterinary Manual, Seventh Edition
2. Coggins, L., Pearson, J. - Diagnostic Services, NVSL, Ames, Iowa; October, 1971. - Protocol for the Immunodiffusion test for EIA.
3. Chong, Y., Payne, S., Issel, C., Montelaro, R., Rushlow, K. - Journal of Virology, Feb. 1991 - Characterization of the Antigenic Domains of the Major Core Protein (p26) of EIA.



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B. EVALUATION OF TEST WELLS -

1. Samples producing color of equal or greater intensity than the Positive Control are POSITIVE for antibodies to EIA.
2. Samples producing color of less intensity than the Positive Control are NEGATIVE for antibodies to EIA.
3. After the test is completed, the Positive Control well can be detached and held next to the test well for easier comparison of color intensity.
4. If desired, results may be read on a microwell plate reader using an air blank. For single wavelength readers, set the wavelength of the plate reader at 630 nm. For plate readers with dual wavelength capability, set the test wavelength at 630 nm and reference wavelength at 490 nm. Samples producing an Optical Density (O.D.) equal to or greater than the O.D. of the Positive Control are POSITIVE for EIA antibodies. Any sample with an O.D. less than the Positive Control is negative.

6. Any questionable sample should be sent to the National Veterinary Services Laboratory (NVSL) for verification. Positive test results should be verified using the Agar Gel Immunodiffusion (AGID) test.

Equine Infectious Anemia Virus Antibody Test Kit

ViraCHEK®/EIA

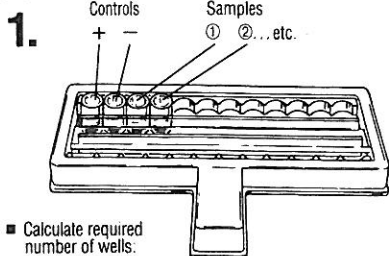
For the
Detection of
Antibodies to
Equine Infectious
Anemia Virus

DIRECTION INSERT

ViraCHEK®/EIA Test Procedure

*NOTE: Only use SERUM samples to perform test.
Prior to use, allow kit components to come to room temperature (70°-78° F; 21°-25° C).*

A. PREPARATION



- 1.**
- Calculate required number of wells.
 - 1 well for positive control
 - 1 well for negative control
 - 1 well for each sample

NOTE: When testing a high number of samples in an assay, Synbiotics strongly recommends including one Negative Control well and one Positive Control well for every 22 samples tested within a run.

- Remove required number of wells.
- Leave wells attached to each other.
- Place in well holder.

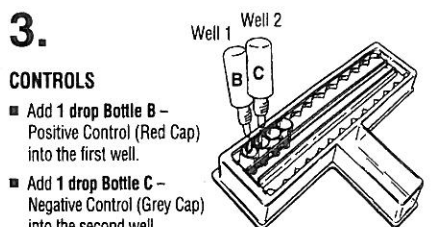
NOTE: If a microwell plate reader will be used to read the results, leave the appropriate space empty so that the plate reader will blank on air.

B. CONJUGATE



- 2.**
- Add 1 drop Bottle A - Conjugate (Blue Cap) into each well.

C. SAMPLE ADDITION

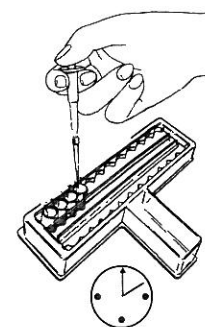


CONTROLS

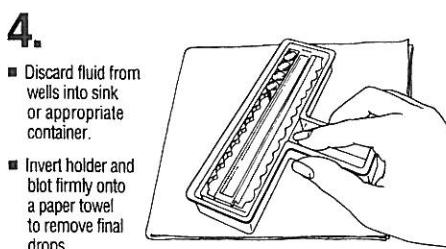
- Add 1 drop Bottle B - Positive Control (Red Cap) into the first well.
- Add 1 drop Bottle C - Negative Control (Grey Cap) into the second well.

SAMPLES

- Pipette 50µl of sample into the next well following the controls.
- Repeat for each additional sample into subsequent wells. Use a separate pipette tip for each sample.
- WAIT 10 MINUTES** (TAP side of well holder for the first 15 seconds of the 10 minute incubation period. Be careful not to splash reagents.)



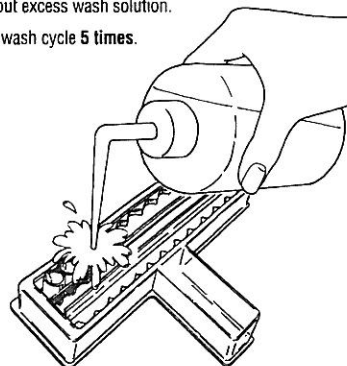
D. BLOT AND WASH



- 4.**
- Discard fluid from wells into sink or appropriate container.
 - Invert holder and blot firmly onto a paper towel to remove final drops.

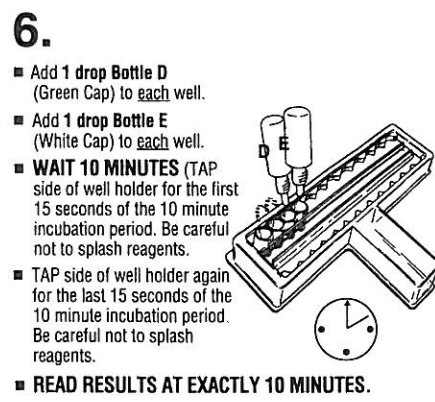
5.

- FLUSH WELLS VIGOROUSLY:**
 - Wash by vigorously filling the wells to overflowing with *diluted wash solution* (See section IV for preparation).
 - Direct a **forceful stream** into each well. (Oversplashing will not contaminate adjacent wells).
 - Shake out excess wash solution.
 - Repeat wash cycle **5 times**.



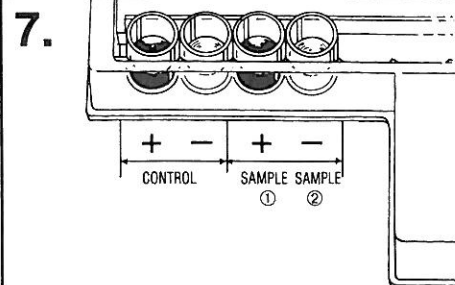
- Wash wells **2 more times** with distilled or deionized water to remove bubbles.
- Blot against a paper towel to dry wells.

E. DEVELOP



- 6.**
- Add 1 drop Bottle D (Green Cap) to each well.
 - Add 1 drop Bottle E (White Cap) to each well.
 - WAIT 10 MINUTES** (TAP side of well holder for the first 15 seconds of the 10 minute incubation period. Be careful not to splash reagents.)
 - TAP side of well holder again for the last 15 seconds of the 10 minute incubation period. Be careful not to splash reagents.
 - READ RESULTS AT EXACTLY 10 MINUTES.**

F. INTERPRETATION OF RESULTS



CONTROLS

- POSITIVE** control should be distinctly blue.
 - NEGATIVE** control should be completely clear.
- NOTE:** If controls appear different than above, repeat the test.

SAMPLES

- POSITIVE** samples will produce color (optical density) equal to or greater than the **POSITIVE CONTROL**.
- NEGATIVE** samples will produce color (optical density) less than the **POSITIVE CONTROL**.

GOOD TECHNIQUES = ACCURATE RESULTS

- Only serum may be used as a sample.
- Hemolyzed and lipemic samples may be used however, severely hemolyzed and lipemic samples may produce background color. When in doubt, obtain a better quality sample.
- Washing is the most important step. Microwells cannot be overwashed.** Underwashing may result in nonspecific blue color development in the negative control and sample wells.
- Prolonged incubation for more than 10 minutes in step 6 may result in non-specific color development.
- Always compare results to the Positive Control. Wells can be detached and compared alongside the Positive Control well against a white background for easier visual inspection.
- Do not use the test kit past the expiration date and do not intermix components from different serial numbers.
- Store kit at 2°-7° C (36°-45° F). Allow kit to come to room temperature before use.

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