DRG® Trichinella spiralis Ab (pig) ELISA (EIA-4838)

Revised 24 Apr. 2008 **For Veterinary Use Only**

INTENDED USE
For the qualitative determination of antibodies in swine sera, plasma, whole blood or tissue fluid to *Trichinella spiralis* using the ELISA technique.

SUMMARY
Trichinellosis is the infection caused by the nematode *Trichinella spiralis*. Although the nematode may be found in a wide variety of animals worldwide, the domestic pig is the primary source of infection in humans in developed nations.[1]
Definitive diagnosis is by demonstration of the larvae in infected muscle tissue. This approach is most successful when there is moderate to high worm burden and maximum larval growth has occurred.
Recently, an excretory-secretory (ES) antigen has been purified from the larvae of infected pigs. This antigen has a high degree of specificity for *T. spiralis*.[2,3] Antibody response will vary depending upon time of infection and worm burden. A low worm burden will cause seroconversion between 28 to 42 days after initial infection. A high worm burden will cause seroconversion between 14 to 28 days.

PRINCIPLE OF PROCEDURE
During the first incubation, the antibodies in the sample binds to the ES antigens in the test well. The next incubation then allows the anti-swine IgG peroxidase complex to bind to the antigen-antibody complex. After a few washings to remove unbound enzyme, a chromogen is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

REAGENTS: (96 Tests)
1. **Test strips:** Microwells containing *T. spiralis* excretory-secretory antigen - Twelve (12) - 8 well strips.
2. **Test strip holder:** One (1) Enzyme conjugate: One (1) bottle containing 11 ml of anti-swine IgG Peroxidase (HRP) in buffer with preservative.
3. **Positive control:** One (1) vial containing 2 ml of diluted *Trichinella* positive swine sera in buffer with preservative.
4. **Negative control:** One (1) vial containing 2 ml of diluted *Trichinella* negative swine sera in buffer with preservative.
5. **Substrate A:** One (1) bottle containing 6 ml of chromogen tetramethybenzidine (TMB).
6. **Substrate B:** One (1) bottle containing 6 ml of citric acid and peroxide.
7. **Wash concentrate (20X) solution:** One (1) bottle containing 25 ml of concentrated buffer and surfactant with preservative.
8. **Dilution buffer concentrate** (20X): One bottle containing 5 ml of concentrated buffered protein solution with preservative.
9. **Stop solution:** One (1) bottle containing 11 ml of 1 M phosphoric acid.

PRECAUTIONS

- Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2-8° C. Crystallization will disappear after dilution to working strength.
- Do not use serum that may have supported microbial growth.
- Do not add azides to the samples or any of the reagents. Controls and some reagents contain a preservative.

STORAGE CONDITIONS

Reagents, strips and bottled components:

- Store between 2 - 8° C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

PREPARATION

Wash Buffer: Remove cap and add 25 ml of Wash Concentrate 20X to 475 ml reagent grade water. Place diluted wash buffer into a squeeze bottle. A narrow tip on the squeeze bottle is recommended to optimize washings. (**NOTE:** Washings consist of filling to overflowing in each well, shaking out the contents and refilling. Using the squeeze bottle at an angle to the well will facilitate proper washing.)

Dilution Buffer: Dilute the Dilution Buffer Concentrate 1:20 in reagent grade water prior to use.

Test samples: Make a 1:200 dilution if using plasma or sera, a 1:100 dilution if using blood and a 1:10 dilution if using tissue fluid.

COLLECTION AND PREPARATION OF SAMPLE

1. Blood (tube with 0.4% sodium citrate), sera, plasma or tissue fluid (by compression of tissue) may be used. Freeze sample at –20° C or lower if not used immediately. See Preparation section (above) for correct sample dilution.
2. Do not heat inactivate sample.
3. Avoid repeated freezing and thawing of samples.

PROCEDURE

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Materials Provided: *Trichinella spiralis* Antibody Test Kit

Materials Required But Not Provided:

1. Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water
4. ELISA plate reader with 450 and 620 to 650 nm filters (optional if results are read visually).
5. Tubes for sample dilutions

PERFORMANCE OF TEST

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 ul of negative control to well #1, 100 ul of positive control to well #2, and 100 ul of the diluted test samples to the remaining wells. **Note:** Negative and positive controls are supplied as prediluted. Use as is.
3. Incubate at room temperature (15 to 25^{o} C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.
5. Add 2 drops (100 ul) of enzyme conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Wash each well once with DI water.
9. Add 1 drop (50 ul) of Substrate A and 1 drop (50 ul) of Substrate B to every well. Mix by tapping strip sideways to vortex reagents. This will produce complete mixing of the solutions.
10. Incubate at room temperature for 10 minutes.
11. Add 2 drops (100 ul) of stop solution.
12. Zero ELISA reader on air, read wells at 450/620 to 650 nm or read results visually.

TROUBLESHOOTING

1. All control wells have excessive color after development:
   Reason: inadequate washings.
   Correction: wash more vigorously.

2. Blue color develops as expected but turns clear upon addition of stop solution:
   Reason: last wash with DI water was not performed.
   Correction: rerun test and make sure a DI water rinse is included before addition of substrates.

Interpretation Of Results - ELISA READER

Zero ELISA reader on air. Read all wells at 450/620 to 650 nm.

- **Positive** - Absorbance reading greater than or equal to 0.3 OD units.
- **Negative** - Absorbance reading less than 0.3 OD units.

Interpretation of Results - VISUAL
Compare results to the controls. A sample should be interpreted as positive if the degree of color development is obvious and significant.

TEST RESULTS

Study #1 - Ohio
Slaughter plant samples: n=11,713
Study compared ARS in-house ELISA versus EIA4838 T. Spiralis Ab ELISA.

	ARS (-)	ARS (+)
EIA4838 T. Spiralis Ab (-)	11,547	2
EIA4838 T. Spiralis Ab (+)	44	120

Sensitivity = 98.4% 95% CI = 94.2% to 99.8%
Specificity = 99.6% 95% CI = 99.5% to 99.7%

Study #2 - Endemic Country for Trichinella
N = 125
Study (ELISA performed at ARS) compared the EIA4838 T. Spiralis Ab ELISA against 100 gram digestion (digestion performed in Romania).

	Digest (-)	Digest (+)
EIA4838 T. Spiralis Ab (-)	22	0
EIA4838 T. Spiralis Ab (+)	0	103

Sensitivity = 100% 95% CI = 84.6 % to 100%
Specificity = 100% 95% CI = 96.5% to 100%

Study #3 - Iowa Slaughter Plant
N = 217,472
Study compared the EIA4838 T. Spiralis Ab ELISA versus 1 gram pooled digestion.

	Digest (-)	Digest (+)
EIA4838 T. Spiralis Ab (-)	217,472	0

EIA4838 T. Spiralis Ab (+)	0	0

Specificity = 100% 95% CI = N/A

Due to the absence of naturally occurring positives in this study site, 31 serum samples from animals with experimental infections (4.2 to 42.9 larvae per gram) were fed into the study as blinded samples. The EIA4838 T. Spiralis Ab ELISA detected 30/31 positive samples. For digestion, 8 meat samples with larvae (4.2 to 42.0 per gram) were also fed into the system as blinded samples. The digestion procedure detected 5/8 samples as positive.

REFERENCES

1. Despommier, D.: Trichinellosis. Immunodiagnosis of Parasitic Diseases, Vol. 1, *Helminthic Diseases*. Ed. Walls and Schantz. Academic Press, 1986. pp. 163-181.
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3. Ivanoska, D., et.al.: Comparative Efficacy of Antigen and Antibody Detection Tests for Human Trichinellosis. *J Parasit*, Vol. 75 #1, 1989, pp. 38-41.
4. Gamble, H. R. and Patrascu, I. V., Whole Blood, Serum, and Tissue Fluids in an Enzyme Immunoassay for Swine Trichinellosis. *J of Food Protection*, Vol. 59, #11, pp. 1213-1217, 1996