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## Certificate of Analysis

### **ANAPLASMA PHAGOCYTOPHILA**

(Formerly *EHRlichia equi*)

FA Substrate Slide

**CATALOG NO.:** 210-88-12-EE

**SIZE:** 12 well

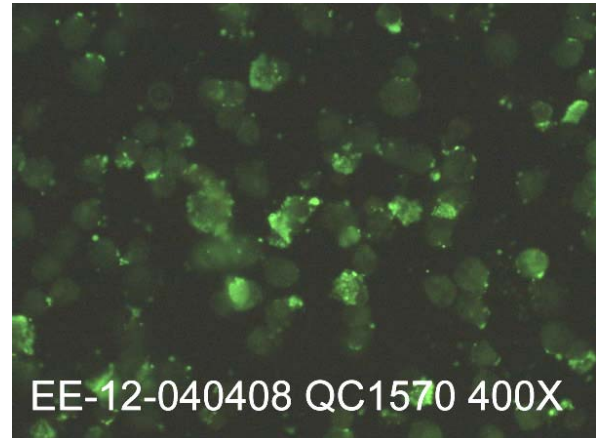
**LOT:** EE-12-040408-041506

**EXPIRATION DATE:** 15 April 2006

**AGENT:** *Anaplasma phagocytophila*

**Cell Culture Substrate:** HL60 cells

**Strain:** NCH-1



**QUALITY CONTROL METHOD:** Indirect FA using *Anaplasma phagocytophila* Positive Control, *Anaplasma phagocytophila* Negative Control, Anti-Equine IgG FITC Conjugate (catalog no. 043-10).

**Specific Reaction:** 3-4+ on positive cells. 30-60 infected cells per high power field, numerous free organisms.

**Other Reactions or Comments:** New lot has much higher infection rate than the reference slide.

**PATTERN OF FLUORESCENCE:** Brightly fluorescent cytoplasmic inclusion bodies (morulae), clustered elementary bodies (initial bodies), and free elementary bodies, approximately 0.3 microns in diameter.

**INTENDED USE:** For detection of antibody to *A. phagocytophila* by indirect FA technique. May be used to differentiate antibody class (IgG or IgM) with suitable quality fluoresceinated second antibody conjugate.

**STABILITY:** Foil-pouch sealed slides are stable until the expiration date when stored at 2-8°C. Avoid self-defrosting freezers.

**DESCRIPTION:** Slides contain fixed *A. phagocytophila* in HL60 cells. Slides are unstained and sealed in moisture-free foil pouches.

**INTERPRETATION OF RESULTS:** Titers of 1:50 (IgG) and greater are considered positive. Sera positive at the 1:50 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same animal.

FOR RESEARCH AND INVESTIGATIONAL USE ONLY.

**WARRANTY:** VMRD, Inc. warrants that this product is as described in the quantity and contents stated on the label at the time of delivery to the customer. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE MADE BEYOND THE LABEL DESCRIPTION, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. Remedy is limited to replacement of the product or refund of the purchase price. VMRD, Inc. is not liable for property damage, personal injury, or economic loss caused by the product. The information listed in this information sheet is provided for reference only, and should not be substituted for the user's own incoming material quality control.

**RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:**

1. Warm slide to room temperature before removing from foil pouch.
2. Place 10 µl diluted serum on the designated wells. Dilute serum (1:50 for screening) in serum diluting buffer, pH 7.2 (catalog no. 210-93-SB). [If background is a problem, particularly at low dilutions, use of 10% adult bovine serum diluting buffer is preferable (catalog no. 210-94-SB).]
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. 210-90-RB) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and flick to remove excess moisture. Place 10 µl labeled anti-IgG or IgM on the wells.
6. Incubate as in step 3.
7. Rinse as in step 4.
8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. 210-92-MF) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

**SERUM DILUTING BUFFER (pH 7.2):\***

- Na<sub>2</sub>HPO<sub>4</sub> ..... 1.19 gm
- NaH<sub>2</sub>PO<sub>4</sub> ..... 0.22 gm
- NaCl ..... 8.55 gm
- BSA ..... 10.0 gm
- DI/dH<sub>2</sub>O ..... Q.S. to 1 liter

\* This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 4°C. Add 0.09% NaN<sub>3</sub> if diluted serum is not going to be used within one week.

**4X FA RINSE BUFFER (pH 9.0):**

- Na<sub>2</sub>CO<sub>3</sub> ..... 11.4 gm
- NaHCO<sub>3</sub> ..... 33.6 gm
- NaCl ..... 8.5 gm
- DI/dH<sub>2</sub>O ..... Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1:4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.