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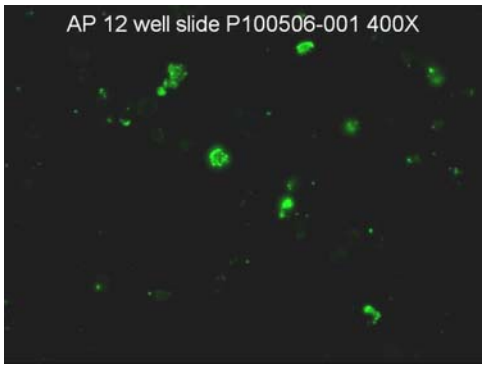
Certificate of Analysis

ANAPLASMA PHAGOCYTOPHILA

IFA Substrate Slide



CATALOG NO.: SLD-IFA-AP
SIZE: 12 Well
LOT: P100506-001
EXPIRATION: 12 May 2012

AGENT: *Anaplasma phagocytophila*
STRAIN: Martin
CELL CULTURE SUBSTRATE: HL-60 cells

DESCRIPTION: Slides contain fixed *A. phagocytophila* in HL-60 cells. Slides are unstained and sealed in moisture-free foil pouches.

QUALITY CONTROL METHOD: Indirect FA using *Anaplasma phagocytophila* Positive Control (catalog no. PC-IFA-AP), *Anaplasma phagocytophila* Negative Control (catalog no. NC-IFA-AP), Anti-Equine IgG FITC Conjugate (catalog no.CJ-F-EQUG-1ML or 10ML).

Specific Reaction: 3-4+ positive fluorescence with the positive control and negative with the negative control with no background. There are 10-50 infected cells per high powered field.

Other Comments: NA

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

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.

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.

STORAGE: Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

REFERENCES: NA

FOR *IN VITRO* LABORATORY USE ONLY.

RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-100ML.
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. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place 50 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. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

RECOMMENDED STAINING PROCEDURE FOR DIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl of direct FA conjugate on the designated wells.
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. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAFM-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

*SERUM DILUTING BUFFER (pH 7.2):**

- Na_2HPO_4 . . . . . 1.19 gm
- NaH_2PO_4 . . . . . 0.22 gm
- NaCl . . . . . 8.55 gm
- BSA . . . . . 10.0 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

* This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7°C. Add 0.09% NaN_3 if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

- Na_2CO_3 . . . . . 11.4 gm
- $NaHCO_3$ . . . . . 33.6 gm
- NaCl . . . . . 8.5 gm
- DI/dH_2O . . . . . 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.