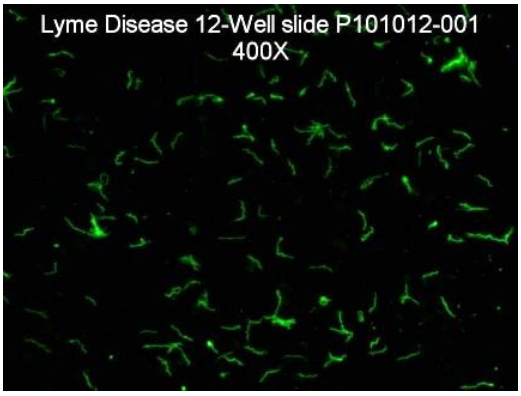
VMRD
PO Box 502, Pullman, WA 99163 USA

Telephone: 509-334-5815
Fax: 509-332-5356
E-mail: vmrd@vmrd.com
Web site: http://www.vmrd.com

Certificate of Analysis

Lyme (*Borrelia burgdorferi*)

IFA Substrate Slide



CATALOG NO.: SLD-IFA-LD
SIZE: 12 Well
LOT: P101012-001
EXPIRATION: 07 December 2012

AGENT: *Borrelia burgdorferi*
STRAIN: B31
CELL CULTURE SUBSTRATE: NA

DESCRIPTION: Slides contain fixed *B. burdorferi* with background suspension. They are unstained and sealed in moisture-free foil pouches.

QUALITY CONTROL METHOD: IFA using VMRD, Inc. Canine Lyme Disease Positive Control (catalog no. PC-IFA-LD), Canine Lyme Disease Negative Control (catalog no. NC-IFA-LD), and Anti-Canine IgG FITC Conjugate (catalog no. CJ-F-CANG-10ML).

Specific Reaction: 4+ fluorescence with the positive control and negative with the negative control and no background. Organisms are too numerous to count per high power field.

Other Comments: NA

PATTERN OF FLUORESCENCE: Spirochetes with uniform surface fluorescence.

INTERPRETATION OF RESULTS: Titers of 1/64 (IgG) and greater are considered positive. Positive control sera, with stated endpoints, are available on request (catalog no. 211-P-LD). Positive titers of 1/256 or less should be confirmed by some other method, i.e., Western Blot Analysis, because of cross-reactions with oral spirochetes [Schillhorn *et al*., Vet. Rec. (1993) 132, p. 512].

INTENDED USE: For detection of antibody to *B. burgdorferi* 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.

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 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 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 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.