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## Certificate of Analysis

### **ROCKY MOUNTAIN SPOTTED FEVER** FA Substrate Slide

**CATALOG NO.:** SLD-IFA-RMSF

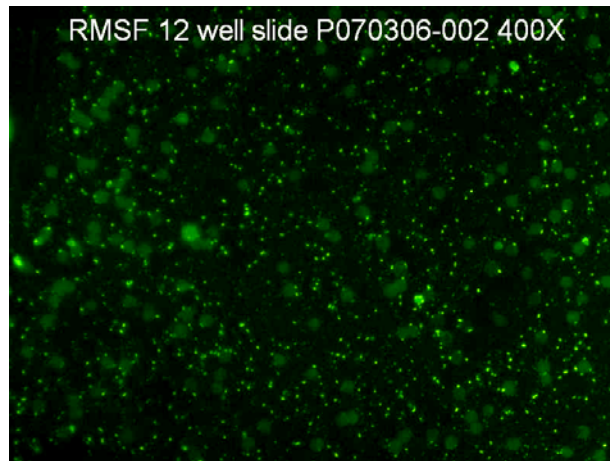
**SIZE:** 12 well

**LOT:** P070306-002

**EXPIRATION:** 30 March 2009

**AGENT:** *Rickettsia rickettsii*

**Strain:** Sheila Smith



**QUALITY CONTROL METHOD:** Indirect FA using VMRD RMSF Positive Control (catalog no. 211-P-RMSF), RMSF Negative Control (catalog no. 211-N-RMSF), Anti-Canine IgG FITC Conjugate (catalog no. 035-10).

**Specific Reaction:** 3-4+ signal with positive control at neat; endpoint at 1:8 dilution; negative with negative control.

**Other Reactions or Comments:** Organisms per high-power field too numerous to count.

**PATTERN OF FLUORESCENCE:** Fluorescent coccobacilli-like structures among ovine red blood cells. Some clumped organisms too numerous to count.

**INTENDED USE:** For detection of antibody to *R. rickettsii* by indirect FA technique.

**STABILITY:** Foil-pouch sealed slides are stable for at least 6 months when stored below 10°C. Avoid self-defrosting freezers.

**DESCRIPTION:** Slides contain formalin, inactivated, fixed *R. rickettsii* cells with ovine red blood cell background to facilitate focusing with negative sera. They are unstained and sealed in moisture-free foil pouches.

**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-RMSF).

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 10 µl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. 210-93-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.