

CANINE RHEUMATOID FACTOR ANTIGEN TEST KIT

CRF®

For The Detection of Canine Rheumatoid Factor

I. INTRODUCTION

Canine rheumatoid arthritis is an erosive polyarthritis frequently characterized by multi-site lameness and joint swelling (Sodikoff, 1981). The disease is often progressive in nature and is distinct from osteoarthritis (degenerative joint disease) and septic arthritis.

The diagnosis of canine RA is difficult. Most authors have chosen to adopt the criteria adopted by the American Rheumatism Association (Shultz, 1977) for the diagnosis of RA in canines. Among these is the presence of rheumatoid factor (RF), an antibody which binds to altered IgG, and which has been reported to be present in 70% of dogs with RA (Sodikoff, 1981).

Several methods have been developed for the detection of canine rheumatoid factor (CRF), among these are the latex agglutination, the modified Rose-Waaler, and the bentonite flocculation procedures. Of these the latex agglutination procedure has been demonstrated to be a simple, stable and consistent test for the presence of rheumatoid factor in dogs (Wood, Hurvitz, and Schultz, 1980).

II. TEST PRINCIPLES

The latex particles provided as a standardized suspension in this kit are coated with canine IgG specifically altered to react with canine rheumatoid factor (CRF). Serum samples are heated in order to inactivate complement factors that may interfere with the test. The coated particles agglutinate when mixed with serum containing CRF, present in rheumatoid arthritis, which recognizes and binds to antigen coated on the latex particles. The agglutination reaction is observed visually, without the need for specialized equipment or instruments. Reagent and sample volumes have been specifically optimized to eliminate the need for predilution of patient sample or references. Do not dilute patient samples or references.

Positive and negative reference sera are provided as a comparative aid to the user in identifying positive or negative reactivity.

III. REAGENTS PROVIDED

1. Canine-Rheumatoid factor latex slide test reagent, 1 vial, 5.0 ml -A suspension of polystyrene latex particles coated with treated canine IgG, standardized to provide proper reactivity in the test procedure. Latex slide test reagent is preserved with sodium azide.
2. RF positive reference serum, 1 vial, 0.5 ml - Serum demonstrates positive reactivity in the latex slide test. RF positive reference serum is preserved with Gentamicin sulfate and Amphotericin B.
3. RF negative reference serum, 1 vial. 0.5 ml Serum which is non-reactive in the latex slide test. RF negative reference serum is preserved with Gentamicin sulfate and Amphotericin B.

IV. WARNING

1. The toxicological properties of these reagents have not been determined. DO NOT INGEST.

2. The kit components contain sodium azide. Upon disposal flush with a large volume of water.
FOR IN VITRO VETERINARY DIAGNOSTIC USE ONLY

V. STORAGE INSTRUCTIONS

Store components at 2 - 7°C. DO NOT FREEZE. These reagents are stable until the expiration date shown on the label.

VI. SPECIMEN COLLECTION

Use only clean serum specimens. The use of plasma is not recommended. Store samples at 2 - 7°C.

VII. MATERIALS REQUIRED

Materials Provided

1. CRF® latex Reagent
2. Positive Reference Serum
3. Negative Reference Serum
4. Glass Reading Slide
5. Dropper
6. 10 µL pipets
7. Stir sticks

Materials Required But Not Provided

1. Water Bath at 56°C
2. Light sources – Tensor lamp or similar
3. Hand magnifier – useful for weak specimens

VIII. ASSAY PROCEDURE

All reagents and serum specimens must be equilibrated to room temperature (18°C - 25°C).

1. a. Place serum samples in 56°C water bath for 30 minutes. Allow samples to cool to room temperature (70 - 78°F) (21 - 25°C) before testing.
b. Using the pipets provided, deposit 10 µL of positive reference into well No. 1.
c. Pipet 10 µL of negative reference into well No. 2.
d. Pipet 10 µL of serum specimens into wells No. 3-6.
2. Mix the CRF latex-reagent by gently swirling the vial contents for about 10-15 seconds. *Do not allow reagent to foam.* Mix the dropper contents by expelling contents into the bulk reagent a few times.
Place 3 drops (approximately 120 µL) of the latex reagent into each well adjacent to the sample of reference serum.
3. Stir the contents of each well with a clean stirring stick. Make sure that the reagent mixes with all of the sample.
4. Rotate the slide evenly in a figure eight pattern for two to three minutes. Examine the slide under a tungsten filament lamp (e.g., Tensor or similar) for latex agglutination.
5. Compare serum samples with the positive and negative reference. Agglutinated reagents indicate positive reactivity; non-agglutinated reagents indicate negative reactivity. The positive reference supplied is adjusted to a mid-range or approximately 2+ reactivity.

IX. NOTES ON PROCEDURE

1. Use only clear serum samples, do not use plasma samples. After blood clotting, serum may be separated by centrifugation or by standard serum filter separator tubes.
2. *Do not allow serum to dry* before addition of latex reagent.
3. Use *only* the proportions of latex reagent and serum specified in the latex Slide Test Procedure. Do not predilute patient sample.
4. After washing slide in detergent, rinse slide thoroughly; residual detergent may affect test results.

Helpful Hints: Pipeting

- Using the microcapillary pipet provided in the kit:
 - a. Insert capillary pipet into the holder provided.
 - b. Allow pipet to draw up patient or reference serum by capillary action. The hole at the top of the rubber bulb must be unobstructed.
 - c. To expel the sample into a well on the glass slide, cover the hole at the top of the black rubber bulb with your index finger and gently squeeze the bulb. The serum will be deposited on the slide.
- It may be helpful to practice this technique a few times with clean water.
- If patient or reference serum is accidentally sucked into the glass barrel of the micropipet holder, disassemble, rinse well with water, dry and reassemble before reuse.
- Do not reuse capillary pipets.
- Other pipeting devices capable of delivering 10 µL and yielding satisfactory results with the positive and negative reference sera may also be used.

X. RESULTS

If latex agglutination occurs in the latex slide test, rheumatoid factor is present in the test serum. Lack of reactivity indicates the absence of rheumatoid factor. Occasionally a slight degree of agglutination may be encountered. Such results may indicate the presence of an early stage of progressive disease and the test should be repeated at a later visit. Increased activity provides evidence for progressive disease. Generally, reactions equal to or greater than the positive reference serum indicate presence of rheumatoid type antibodies. Such findings combined with the criteria in section XIII assist in the diagnosis of rheumatoid arthritis in the canine.

XI. LIMITATIONS OF THE PROCEDURE

1. Use only clear serum samples; plasma or lipemic samples may non-specifically agglutinate latex particles.
2. Store the latex slide reagent with the cap tightly closed to prevent evaporation of the buffered liquid.
3. If the glass slide is rotated for more than five minutes, false positive results may occur.

XII. PERFORMANCE CHARACTERISTICS

1. Sera from a group of healthy dogs were found to exhibit negative reactivity when tested by the Canine Rheumatoid Factor Latex Slide Test.
2. Sera from a group of dogs exhibiting rheumatoid factor as determined by a qualified clinical laboratory performing veterinary rheumatoid factor determinations were found to exhibit positive reactivity in the Canine Rheumatoid Factor Latex Slide Test.

XIII. HELPFUL HINTS

Utilizing criteria established in humans and accepted by the American Rheumatoid Association the following criteria might be helpful, as quoted from Berkow, *et al.*¹:

1. Diagnostic Criteria for Rheumatoid Arthritis:
 - a. **Classic Rheumatoid Arthritis**

This diagnosis requires seven of the following criteria. In criteria 1 through 5 the joint signs or symptoms must be continuous for at least 6 wk.

 - 1) Morning stiffness.
 - 2) Pain on motion or tenderness in at least one joint (observed by a physician).
 - 3) Swelling (soft tissue thickening or fluid, not bony overgrowth alone) in at least one joint (observed by a physician).
 - 4) Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms between the two joint involvements may not be more than 3 mo).

- 5) Symmetric joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of proximal interphalangeal, metacarpophalangeal, or metatarsophalangeal joints is acceptable without absolute symmetry). Terminal phalangeal joint involvement will not satisfy this criterion.
- 6) Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces, or in juxta-articular regions.
- 7) X-ray changes typical of rheumatoid arthritis (which must include at least bony decalcification localized to or greatest around the involved joints and not just degenerative changes). Degenerative changes do not exclude patients from any group classified as rheumatoid arthritis.
- 8) Positive agglutination test - demonstration of the rheumatoid factor by any method which, in two laboratories, has been positive in not >5% of normal controls.
- 9) Poor mucin precipitate from synovial fluid (with shreds and cloudy solution).
- 10) Characteristic histologic changes in synovial membrane with three or more of the following: marked villous hypertrophy; proliferation of superficial synovial cells, often with palisading; marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating), with tendency to form "lymphoid nodules"; deposition of compact fibrin either on surface or interstitially; foci of cell necrosis.
- 11) Characteristic histologic changes in nodules showing granulomatous foci with central zones of cell necrosis, surrounded by a palisade of proliferated macrophages, and peripheral fibrosis and chronic inflammatory cell infiltration, predominantly perivascular.

b. Definite Rheumatoid Arthritis

This diagnosis requires five of the above criteria. In criteria 1 through 5 the joint signs or symptoms must be continuous for at least 6 wk.

c. Probable Rheumatoid Arthritis

This diagnosis requires three of the above criteria. In at least one criteria 1 through 5 the joint signs or symptoms must be continuous for at least 6 wk.

d. Possible Rheumatoid Arthritis

This diagnosis requires two of the following criteria and total duration of joint symptoms must be at least 3 wk.

- 1) Morning stiffness.
- 2) Tenderness or pain on motion (observed by a physician) with history of recurrence or persistence for 3 wk.
- 3) History or observation of joint swelling.
- 4) Subcutaneous nodules (observed by a physician).
- 5) Elevated ESR or C-reactive protein.
- 6) Iritis (of dubious value as a criterion except in the case of juvenile rheumatoid arthritis).

XIV. REFERENCES

1. Berkow, MD. R. (Editor), 1977. The Merck Manual of Diagnosis and Therapy - Thirteenth Edition. Merck Sharp Dohme Research Laboratories-Division of Merck & Co., Inc., Rahaw, NJ.
2. Schultz, A.O., 1977. Laboratory Diagnosis of Immunological Diseases. In Kirk, R. W. (Editor). Current Veterinary Therapy VI. W. B. Saunders, Co., Philadelphia, PA.
3. Sodikoff, C., 1981. Laboratory Profiles of Small Animal Disease. American Veterinary Publications, Inc., Santa Barbara, CA.
4. Wood, D. D., Hurvitz, A. I. and Shultz, R. D., 1980. A Latex test for canine rheumatoid factor. Vet. Immunol Immunopathol., 1:103-111.