



Anaplasma / Ehrlichia MIF

Performance Characteristics

The indirect immunofluorescence antibody assays (IFA) for *Ehrlichia spp.* and *Anaplasma phagocytophilum* have been used for many years as gold standards for serodiagnosis. In this format both live purified antigens are robotically placed within each slide well with a background matrix. The matrix is added to make the antigen location visible when there is no fluorescence (negative reaction).

Anaplasma Sensitivity

For *Anaplasma* testing, the initial Fuller Laboratories IFA was introduced in 1995 and utilized the MRK equine strain of *A. phagocytophilum* grown in KG-1 cells. Comparison between this former substrate and the current NCH-1 isolate grown in HL60 cells (25 positive and 25 negative sera) demonstrates 100% concordance.

Correlations of Anaplasma IFA protocols with Western Immunoblot (WB) techniques demonstrate IFA sensitivity between 80-100%³. An in-house series of 48 dog sera from the state of New York showed complete (100%) concordance between IFA and WB on 20 positive sera and 28 negative.

Ehrlichia Sensitivity

The IFA for *Ehrlichia canis* was described in the literature in 1972⁶. The Fuller Laboratories test uses purified Oklahoma/LSU isolate, propagated in the DH82 canine macrophage cell line, as substrate.

Antibody detection becomes detectable at approximately the time most dogs begin showing clinical signs, 21-40 days post infection¹⁻². Due to the wide variety of antigen

present on the whole organism by the IFA technique, sensitivity is approximately equal to Western immunoblot assay using whole cell lysates²⁻⁵.

Anaplasma Specificity

With the incorporation of *Ehrlichia equi*, *Ehrlichia phagocytophila* and the HGE Agent into the species *Anaplasma phagocytophila* comb. nov. , there are few close relatives to this combined species. Low level cross-reactivity may be seen with *Anaplasma platys* by IFA. There have been reports of human serum crossreactivity of *Anaplasma* with *Ehrlichia chaffeensis*, but they have not been recorded for this particular assay format. There are no sources of crossreactivity outside the tribe Ehrlichiae.

Canine sera from a non-endemic region, metropolitan Southern California, were tested as a source of negative sera. Of 58 sera tested, all (58/58) were negative (100% specificity) for Anaplasma.

Ehrlichia Specificity

Ehrlichia canis crosses strongly with *Ehrlichia chaffeensis*, *Ehrlichia ewingii* and possibly other Ehrlichia spp. With the MIF technique there is no cross-reactivity with *Anaplasma*.

Sera from non-endemic regions were tested in-house, 159 from New York and 120 from Southern California. There were no positives (100% specific). As the New York sera were from an endemic region for *Anaplasma phagocytophila*, there were found 14 dogs (8.8%) seropositive for this related organism.

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