**Anaplasma phagocytophilum**

**IFA IgG (OUS)**

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<th>IF1450G</th>
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Indirect Immunofluorescent Assay (IFA) for the Detection of Human IgG Antibodies to *A. phagocytophilum*

This package insert is for export only and not for distribution in the United States.

**Outside of the United States:**

For in vitro Diagnostic Use.

**INTENDED USE**

The Focus Diagnostics *Anaplasma phagocytophilum* Indirect Immunofluorescence Antibody (IFA) IgG test is intended for the detection and semi-quantitation of human serum IgG class antibodies to *Anaplasma phagocytophilum*, as an aid in the diagnosis of Human Granulocytic Anaplasmosis (HGA).

**SUMMARY AND EXPLANATION OF TEST**

In 2001, Dumler et al recommended classifying the agent that causes HGA as *Anaplasma phagocytophilum*. After review of the nomenclature, *Anaplasma phagocytophilum* was accepted as the proper name. The genera *Anaplasma*, *Ehrlichia*, *Cowdria*, *Neorickettsia* and *Wolbachia* encompass a group of bacteria that were previously classified based upon morphological, ecological, epidemiological and clinical characteristics. Instead, recent genetic analyses have indicated that the genera should be grouped roughly as follows:

1. *Anaplasma* (including the *Ehrlichia phagocytophila* group, *Ehrlichia platys* and *Ehrlichia bovis*)
2. *Ehrlichia* (including *Cowdria ruminantium*)
3. *Wolbachia*
4. *Neorickettsia* (including *Ehrlichia sennetsu* and *Ehrlichia risticii*)

Insufficient differences existed among *E. phagocytophila*, *Ehrlichia equi* and the human granulocytic ehrlichiosis (HGE) agent to support separate species designations.

*A. phagocytophilum* was first discovered after a number of patients in Minnesota and Wisconsin were observed with an acute febrile illness between 1990 and 1993. While microscopic examination revealed membrane-bound microcolonies (morulae) in the granulocytes of these patients, initial efforts to culture the organisms were unsuccessful. Chen et al. identified the agent as a member of the *E. phagocytophila* genogroup using PCR, followed by its isolation in HL60 cells by Goodman et al.

Human Granulocytic Anaplasmosis is a zoonotic disease transmitted by nymphaal and adult ticks: the deer tick *Ixodes scapularis* in the United States and *Ixodes ricinus* in Europe. In the U.S. *A. phagocytophilum* has been recognized in the upper Midwest, the Northeast and Northern California. In Europe seropositivity rates range from 3 to 17% of patients reporting *Ixodes* tick bites from Italy, Sweden and Switzerland. Coinfection with *Borrelia burgdorferi*, the etiologic agent of Lyme disease, is possible due to a common arthropod vector.

The clinical manifestations of HGA in most patients are first revealed as flu-like symptoms, including fever, myalgia, rigors, and headaches; rashes are seldom observed. Laboratory assessment reveals mildly elevated aspartase aminotransferase, lactate dehydrogenase, creatinines and erythrocyte sedimentation rate. Bakken et al. have reported an inverse relationship between the probability of HGA infection and the values of absolute white blood cell (WBC) and/or platelet counts. HGA responds favorably to treatment with doxycycline or tetracycline, with defervescence occurring within 48 hours and complete recovery within seven days.

HGA patients often show a robust immune response to *A. phagocytophilum*. In the absence of treatment, detectable IgM levels generally rise 3 to 5 days post infection or 24 hours after the initial onset of fever, falling again to undetectable levels in about 30 to 60 days. IgG levels often are detectable about 7 to 10 days post infection, peaking at 14 to 21 days and persisting for approximately a year. Seropositivity rates for both IgG and IgM antibodies tend to peak in July, August and September.

Since the *A. phagocytophilum* is observed in peripheral granulocytes in only 20% of patients studied, it cannot be depended on as a sensitive diagnostic test. Serological methods, including Western blot and indirect immunofluorescence assays (IFA) are more reliable. The most commonly used technique for HGA diagnosis is IFA which should include both IgG and IgM specific antibody screens for maximal certainty.

The Focus Diagnostics *Anaplasma phagocytophilum* IFA IgG assay utilizes HGE-1 strain infected HL60 cells. Each slide contains twelve wells.

**TEST PRINCIPLE**

The indirect immunofluorescent antibody (IFA) assay is a two stage “sandwich” procedure. In the first stage, the patient serum is diluted in PBS. The diluted serum is placed on the slide in contact with the substrate, and incubated. Following incubation, the slide is washed in PBS, which removes unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labeled antibody to human IgG. The slide is incubated allowing antigen antibody complexes to react with the fluorescein-labeled anti-human IgG. After the slide is washed, dried, and mounted, it is examined using fluorescence microscopy. Positive reactions appear as apple-green fluorescence of the morulae. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.
Anaplasma phagocytophilum IFA IgG Test Kit contains sufficient materials to perform 120 determinations.

**MATERIALS SUPPLIED**

Focus Diagnostics’ *Anaplasma phagocytophilum* IFA IgG Test Kit contains sufficient materials to perform 120 determinations.

**IgG Substrate Slide**

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10 slides of twelve wells. Each well contains inactivated cells infected with *A. phagocytophilum*. The sealed slides are stable until the date stated on the slide packet labels when stored at 2 to 8°C. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.

**IgG Conjugate, 3.3 mL**

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One vial of affinity purified and fluorescein-labeled goat anti-human IgG gamma-chain specific. Contains Evan’s Blue counterstain, protein stabilizer and preservatives. Ready for use. Stable until the date stated on the label when stored at 2 to 8°C. Allow to warm to room temperature before use. Repeated freezing and thawing is deleterious and should be avoided.

**IgG Detectable Control, 0.3 mL**

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One vial of human serum bottled at screening dilution. Contains preservatives. The control is stable until the expiration date stated on the label, when stored at 2 to 8°C. For long term storage, aliquot in small volumes (25μL) and store at ≤70°C. Do not use if cloudiness, discoloration or other indication of bacterial contamination are present. Allow to warm to room temperature before use. Repeated freezing and thawing is deleterious and should be avoided.

**Non-Detectable Control, 0.25 mL**

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One vial of human serum bottled at screening dilution (representing a screening dilution of 1:64). Contains preservatives. Stable until the expiration date stated on the label, when stored at 2 to 8°C. Do not use if cloudiness, discoloration or other indication of bacterial contamination are present. Allow to warm to room temperature before use. Do not dilute. Repeated freezing and thawing is deleterious and should be avoided.

**Mounting Medium, 2.5 mL**

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One dropper bottle containing PBS-buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. Stable until the expiration date stated on the bottle labels, when stored at 2 to 8°C. Allow to warm to room temperature before using.

**PBS**

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One vial of phosphate buffered saline (PBS) powder. Reconstitute with 1 Liter distilled (or purified) water. The reconstituted solution is a 0.01M buffer at pH 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before use. Do not use if cloudiness, discoloration, or other indication of bacterial contamination are present.

**MATERIALS REQUIRED, BUT NOT SUPPLIED**

1. 24 x 50 mm coverslips
2. Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions
3. Clinical centrifuge
4. 35 to 37°C incubator or waterbath for slide incubation
5. 2 to 8°C refrigerator
6. Plastic wash bottle
7. Calibrated pipets or piston-type pipettors with disposable tips
8. Coplin jars or slide staining dish with slide holder
9. Clean volumetric flask or graduated cylinder, 1 liter
10. Humid chamber for incubation of slides
11. Distilled or purified water
12. Timer
13. Absorbent paper for blotting slides
14. Fluorescence microscope, recommended parameters
    - Excitation Filter: 470 – 490 nm
    - Barrier Filter: 520 – 560 nm
    - Light Source: HBO 100W, mercury
    - Objective: 20 – 40X, fluorescence quality, high dry

**SHELF LIFE AND HANDLING**

1. Kits are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.

**WARNINGS AND PRECAUTIONS**

1. This package insert is for export only and not for distribution in the United States. Outside of the United States this kit is for *in vitro* diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including non-detectable controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions.  
3. The substrate slides contain inactivated cells infected with *A. phagocytophilum*. However, the slides should be considered potentially infectious and handled accordingly.
4. Evan’s Blue is a carcinogen; however this product is below the reportable threshold (less than 0.1%).
5. Do not substitute or mix reagents from different kit lots or from other manufacturers.
6. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
7. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle slides carefully to avoid mixing of sera from adjacent wells.
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
9. Mounting Medium contains 30 to 60 % glycerol which may cause irritation upon inhalation or skin contact. Upon inhalation or contact, first aid measures should be taken.

**SPECIMEN COLLECTION AND PREPARATION**

Serum is the preferred specimen source. No attempt has been made to assess the assay’s compatibility with other specimens. Hyperlipemic, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

**Specimen Collection and Handling**

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen at −20°C or colder. Freeze-thaw damage can result if specimens are frozen in self-defrosting freezers. Thaw and mix samples well prior to use.

**Specimen Preparation**

The serum screening dilution is 1:64 in PBS.

Where it is necessary to determine endpoint titers, use reconstituted PBS to serially dilute the final screening dilution.

**TEST PROCEDURE**

1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening the slide packets.
2. Apply 25 μL of IgG Detectable Control, as bottled, to the appropriate well. Use PBS to perform serial 2-fold dilutions of the Detectable Control to a 1:32 dilution. Apply 25 μL of each serial dilution to an appropriate slide well.
3. Apply 25 μL of Non-Detectable Control, as bottled, to the appropriate well. Do not dilute the Non-Detectable Control.
4. For each patient sample to be tested, add approximately 25 μL of the prepared sample dilutions (see Specimen Preparation, above) to an appropriate slide well. Make notations to later identify each well when reading the results.
5. Incubate slide(s) in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
6. Remove slides from the humid chamber and gently rinse each slide with a stream of PBS. Do not aim the stream of PBS directly at the slide wells. Rinse one row at a time to avoid mixing of specimens. Wash slides by submerging the rinsed slides into Coplin or slide staining jars containing PBS for 10 minutes.
7. Dip the washed slides briefly in distilled or purified water, and allow the slides to air dry.
8. Add approximately 25 μL IgG Conjugate to each slide well.
9. Incubate slides in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
10. Repeat wash steps 6 and 7.
11. Place a few drops of Mounting Medium on the slide and cover with a 24 x 50 mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbent paper.
12. View wells at a final magnification of 400X on a properly equipped fluorescence microscope. For optimal fluorescence, read slides the same day the assay is performed. If this is not possible, store in the dark at 2 to 8°C up to 24 hours.

**QUALITY CONTROL**

Each run (each time a slide, or group of slides, is processed) should include both Detectable and Non-Detectable controls.

1. The Detectable Control should exhibit fluorescence at 8-fold beyond the bottled dilution. However, due to differing laboratory conditions including equipment, the endpoint may range from 4 to 16-fold beyond the bottled dilution.
2. The Non-Detectable Control should exhibit negligible reactivity to all spots. Fluorescence that does not match the morphology and distribution of the detectable control is considered not detectable.

If controls do not exhibit these results, patient test results should be considered invalid and the assay repeated.

**INTERPRETATION OF TEST RESULTS**

Microscopic optics, light source condition, and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.

Read the fluorescent intensity of the morula on each spot, and grade the fluorescence as follows:

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<tr>
<th>2 to 4+</th>
<th>Moderate to intense apple-green fluorescence of the morula.</th>
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<tr>
<td>I+</td>
<td>Definite, but dim fluorescence equivalent to that observed for the Detectable Control at its reference endpoint titer.</td>
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<tr>
<td>Negative</td>
<td>No Fluorescence of fluorescence equal to that observed in the Non-Detectable Control well (or less than endpoint titer).</td>
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**Interpreting the Patient Specimen Results**

The reciprocal of the highest serum dilution that exhibits definite (I+) apple-green fluorescence of the morulae is termed the serum endpoint titer.

| ≥1:64 | Single IgG serum endpoint titers ≥1:64 are suggestive of infection at an undetermined time and may be indicative of either past infection or early response to a recent infection. |
|<1:64 | No antibody detected. |

A four-fold or greater increase in IgG titer between two serum samples drawn 1 to 2 weeks apart and tested in parallel is considered presumptive evidence of recent or current infection by the HGA agent.

**LIMITATIONS**

1. It is essential that all results from *A. phagocytophilum* serologies correlate with clinical history and other data available to the physician.
2. Microscope optics and light source condition and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.
3. Diagnostic antibody levels may persist for at least three years. An IgG endpoint greater than 1:64 alone should not be relied on for an Anaplasmosis diagnosis.

**EXPECTED VALUES**

Seropositivity rates ranged from 3 to 17% of patients reporting Ixodes tick bites in Italy, Sweden and Switzerland.

*Anaplasma phagocytophilum* IFA IgG

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PERFORMANCE CHARACTERISTICS
For customers outside of the United States, the product performance characteristics are supplied as a separate sheet.

REFERENCES
10. CDC. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. (2007).

This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and may be available in other languages from your local distributor.