Performance Characteristics

**SPECIFICITY**

Two external investigators and an internal investigator evaluated specificity with a total of 85 sera: 60 sera from non-endemic areas and 25 sera from an endemic area. All sera were from unexposed blood donors. The Focus *Anaplasma phagocytophilum* IFA IgM was negative with 100% (45/45) of the endemic and non-endemic European sera. Of the non-endemic U.S. sera, the Focus *Anaplasma phagocytophilum* IFA IgM was negative with 97.5% (39/40).

**Specificity with Blood Donors**

<table>
<thead>
<tr>
<th>Population</th>
<th>% Negative</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic (Slovenia)</td>
<td>100%</td>
<td>(25/25)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86.3-100%</td>
</tr>
<tr>
<td>Non-endemic (France)</td>
<td>100%</td>
<td>(20/20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83.2-100%</td>
</tr>
<tr>
<td>Non-endemic (U.S.)</td>
<td>97.5%</td>
<td>(39/40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86.8-99.9%</td>
</tr>
</tbody>
</table>

† One sample scored negative did have some non-specific reactivity.

**SENSITIVITY**

**Sensitivity with PCR/Blood Smear Positives by Time**

An external investigator evaluated sensitivity using thirty sera from fifteen patients that were diagnosed with *Anaplasma phagocytophilum* infections. *Anaplasma phagocytophilum* infection was diagnosed based on the following criteria:

1) acute febrile illness, and
2) *Ixodes* species tick bite in an endemic area, and
3) laboratory confirmed (positive by PCR and/or blood smear).

The PCR was specific for 16S rDNA of *Anaplasma phagocytophilum*, and was described by Chen, et al. Blood smears were positive if intracytoplasmic morulae were identified in blood, bone marrow, or CSF. Sensitivity was stratified based on days after onset of symptoms. Of the thirty samples, 14 were drawn less than 60 days after symptom onset, 13 were drawn greater than 60 days, and three were drawn an unknown number of days after symptom onset. The Focus *Anaplasma phagocytophilum* IFA IgM had the following sensitivity:

- **Less than 60 days**: of the fourteen drawn less than 60 days after symptom onset, 71.4% (10/14) were positive.
- **More than 60 days**: of the thirteen drawn more than 60 days after symptom onset, 7.7% (1/13) were positive.
- **Unknown**: of the three sera drawn at an unknown number of days after symptom onset, 100.0% (3/3) were positive.

**Sensitivity with Paired Sera**

An external investigator evaluated sensitivity using 15 pairs of sera drawn at least two weeks apart from 15 patients that were diagnosed with *Anaplasma phagocytophilum* infections. *Anaplasma phagocytophilum* infection was diagnosed based on the following criteria:
1) clinical symptoms compatible with Anaplasma phagocytophilum infection and
2) a four-fold or greater change in the investigator's in-house Anaplasma phagocytophilum IFA IgG titer.

The clinical symptoms included febrile illness, history of tick bite, leukopenia and thrombocytopenia and/or elevated hepatic transaminase. The investigator's in-house IgG IFA was previously described by Nicholson, et al. Of the 15 pairs of sera, 11 pairs demonstrated a four-fold rise with the investigator's in-house IgG IFA (acute), and four pairs demonstrated a four-fold fall in the investigator's in-house IgG IFA (convalescent). Of the 11 pairs demonstrating a four-fold rise, the Focus Anaplasma phagocytophilum IFA IgM was positive for 9.1% (1/11) with the first draws, and 72.7% (8/11) with the second draws. Of the four pairs demonstrating a four-fold fall in the investigator's in-house IFA, the Focus Anaplasma phagocytophilum IFA IgM was positive with none of the first draws and none of the second draws.

### CROSS-REACTIVITY

Two external investigators evaluated cross-reactivity by selecting sera from patients that were symptomatic for and sero-positive for the potential cross-reactant.

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>% Positive</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tickborne Encephalitis</td>
<td>0.0%</td>
<td>(0/15) 0.0-21.8%</td>
</tr>
<tr>
<td>Mediterranean Spotted Fever (Rickettsi conorii)</td>
<td>0.0%</td>
<td>(0/25)</td>
</tr>
<tr>
<td>Acute Q Fever (Coxiella burnetii)</td>
<td>0.0%</td>
<td>(0/10)</td>
</tr>
<tr>
<td>Lyme Borreliosis (Borrelia burgdorferi)**</td>
<td>0.0%</td>
<td>(0/36)</td>
</tr>
<tr>
<td>Bartonella quintana</td>
<td>0.0%</td>
<td>(0/10) 0.0-41.0%</td>
</tr>
</tbody>
</table>

** Co-infection with both Borrelia burgdorferi and Anaplasma phagocytophilum have been reported.3

### STABILITY

An internal investigator evaluated stability. Components were accelerated to a 2 to 8°C equivalent of one and two years. The accelerated components were then tested according to the package insert in parallel with unaccelerated components. Components are considered stable if they meet the QC criteria specified in the package insert. The product was found stable after accelerating for both one and two years.

### REPRODUCIBILITY

#### Intra-assay and Inter-assay Reproducibility

An internal investigator evaluated intra-assay and inter-assay reproducibility. Ten patient sera were run on three separate slides each day over five days (n = 150). Four sera were positive, one was borderline and five sera were negative. Results for all sera were within one two-fold dilution.

#### Inter-lot Reproducibility

An internal investigator evaluated inter-lot reproducibility. Ten patient sera were run on three lots of slides (n = 30). Four sera were positive, one was borderline and five sera were negative. Results for all sera were within one two-fold dilution.

### REFERENCES


### ORDERING INFORMATION

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