IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

### References

### Materials Provided
- Wash concentrate 20X: 1 bottle
- Stop Solution: 1 bottle (ready to use)
- TMB Substrate: 1 bottle (ready to use)
- Enzyme conjugate: 1 bottle (ready to use)
- Positive Control: 1 vial (ready to use)
- Negative Control: 1 vial (ready to use)
- Sample Diluent: 22 ml
- Microwell coated with M. pneumoniae antigen: 12x80X
- Calibrator: 1 Vial (ready to use)
- Wash concentrate 20X: 1 bottle

### Materials Not Provided
- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph paper

### INTENDED USE
The Mycoplasma pneumoniae (M. pneumoniae) IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to M. pneumoniae in human serum or plasma. For research use only.

### SUMMARY AND EXPLANATIONS
Mycoplasma pneumoniae is a pathogen with spectrum of clinical presentations ranging from asymptomatic to pronounced pneumonia. Symptoms start from 6 to 32 days after exposure with headache, malaise, cough, sore throat and fever. The illness can last from a few days to a month or more. Detection by ELISA of M. pneumoniae IgM antibodies or demonstration of a significant increase of specific IgG antibodies is strong evidence for recent infection in the appropriate clinical setting. Specific IgM antibodies typically increase significantly 1 week after clinical onset and specific IgG levels rise in the second week. M. pneumoniae IgM can, however, persist for more than two years after infection, and therefore, detection of specific IgM does not accurately indicate the time of infection. Primary infection and reinfection may be distinguished by the presence of elevated specific IgA and of specific IgM in primary infections and by the presence of elevated specific IgA in the absence of specific IgM in reinfections. In general, the absence of specific IgM in serum collected 10-20 days after onset is strong evidence against primary pneumonia due to M. pneumoniae.

### Principles of the Test
Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.
STORAGE AND STABILITY
1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is for research use only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush the calibrator factor (CF) = 0.5

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Standard should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION
The following is intended as a guide to interpretation of M. pneumoniae IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation
<0.9 No detectable antibody to M. pneumoniae IgG by ELISA.
0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 Detectable antibody to M. pneumoniae IgG by ELISA.

LIMITATIONS OF THE TEST
1. The test results obtained using this kit are for research use only and are not intended to be used as a part of any official diagnosis.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
47 patient sera were tested by this M. pneumoniae IgG ELISA and a reference ELISA method. 109 sera were positive and 31 were negative by both methods (95% agreement). The results are summarized below:

<table>
<thead>
<tr>
<th>M. pneumoniae IgG ELISA</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ELISA Kit</td>
<td>109</td>
<td>3</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>35</td>
<td>147</td>
</tr>
</tbody>
</table>

2. Precision
Intra-Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.77</td>
<td>0.08</td>
<td>4.5</td>
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<tr>
<td>2</td>
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<td>0.97</td>
<td>0.06</td>
<td>6.2</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.15</td>
<td>0.01</td>
<td>6.6</td>
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