ImmunoCard® MYCOPLASMA

A Rapid Enzyme Immunoassay for the Detection of IgM to *Mycoplasma pneumoniae* in Human Serum

REF **709030** IVD **Rx Only**

INTENDED USE
The Immuno*Card* Mycoplasma enzyme immunoassay (EIA) is an in vitro qualitative procedure for the detection of IgM to *Mycoplasma pneumoniae* in human serum. Test results are intended to aid in the diagnosis of recent *Mycoplasma pneumoniae* infection.

SUMMARY AND EXPLANATION OF THE TEST
Mycoplasma pneumoniae is a member of a group of degenerate bacteria lacking a cell wall.[1] *M. pnuemoniae* was the first human pathogen identified in the group and causes up to 20% of all cases of pneumonia.[1,2] Mycoplasmal pneumonia presents with flu-like symptoms,[3] however unlike most viral and bacterial pneumonias, mycoplasmal pneumonia is more gradual in both presentation and recovery. *M. pneumoniae* infections are usually grouped in the category of atypical pneumonia. Examples of other organisms which cause atypical pneumonia are influenza (A and B), respiratory syncytial virus, adenovirus, parainfluenza, cytomegalovirus, *Chlamydia, Legionella, Histoplasma capsulatum* and *Coccidioides immitis*.[3] *M. pneumoniae* disease progression is usually limited to the respiratory system from the naso-pharynx through bronchioles; resulting in widely varying symptoms more consistent with bronchitis than pneumonia. Antibiotics may ameliorate symptoms, however organism can often be cultured from patients following antibiotic regimes. Asymptomatic (silent) infections may occur in adults and account for up to 20% of *M. pneumoniae* infections.[4]

M. pneumoniae is endemic, with minimal seasonal variation (small increases in the late summer/early fall).[2,5] Incidence overall ranges from 0.5 to 5.0 per 1,000 population or up to 20% of all pneumonias. Incidence peaks with 5-9 year olds and declines with age except for a slight rise in the 30-40 year age group. The disease is rare in adults over 50 and infants, although the impact may be severe in these groups. The organism appears to require close contact for transmission. Development of symptoms may take several weeks, and transmissible organism may persist once symptoms have subsided. Epidemics occur in 4 to 7-year cycles worldwide and may be linked to childhood school and day-care facilities.[5,6]

Direct detection of *Mycoplasma pneumoniae* infection is currently difficult, owing the slow growth (4-20 days) of the organism in culture and fastidious growth requirements.[1] For this reason, serology is often the best laboratory method available.[7,8] The complement fixation (CF) test identifies antibody to a mycoplasmal lipopoly saccharide (LPS). In general, laboratories suggest that four-fold increases in CF titer using paired acute/convalescent sera, or CF titers ≥ 64 are diagnostic. Other serological tests include enzyme immunoassays (EIAs) and immunofluorescent assays (IFAs) for the detection of IgG or IgM, and detection of cold agglutinins. The advantage of an IgM based assay is the detection of early/acute illness rather than convalescent disease where the switch to IgG has occurred.

The presence of IgM to *M. pneumoniae* is considered to have a role in the diagnosis of early/acute disease.[4,9,10,11] Tests which detect both IgM and IgG or tests which detect IgG only have the problem of being positive in convalescent patients, as well as in individuals with previous history of *M. pneumoniae* disease (subclinical).

The Immuno*Card* Mycoplasma methodology provides a simple to use, self-contained device. No calculations are required and the visual color change makes interpretation of results objective and simple.

BIOLOGICAL PRINCIPLES
The Immuno*Card* Mycoplasma EIA detects the presence of IgM to *M. pneumoniae* in serum. Patient serum is added to each of the two sample ports. After allowing the sample to enter the device and migrate along the membrane and through the reaction ports, three drops of anti-human IgM alkaline phosphatase conjugate are added to the sample ports and allowed to enter the device. Three drops of wash and two drops of substrate are then added to each of the reaction ports. Reaction ports are observed for the development of any blue color after five minutes. The **CONTROL** port serves as a procedural control, containing immobilized human IgM in the reaction port. The **TEST** port contains *M. pneumoniae* antigens and serves as the patient test port. The development of blue color in the **TEST** port indicates a reactive test result for IgM to *M. pneumoniae*. No blue color in the **TEST** port indicates a nonreactive result.

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. **Test Cards** - Individually foil pouched cards containing immobilized detergent extracted *M. pneumoniae* antigens (**TEST** reaction port) and human IgM (**CONTROL** reaction port)
2. **Positive Control** - Sample containing human anti-*M. pneumoniae* IgM in a buffer containing 0.1% sodium azide
3. **Negative Control** - Buffer containing 0.1% sodium azide
4. **Enzyme Conjugate** - Monoclonal anti-human IgM labeled with alkaline phosphatase in a buffer containing 0.1% sodium azide
5. **Wash Buffer** - Buffer containing 9.5% (weight/vol.) guanidine hydrochloride
6. **Substrate Reagent** - Buffered solution containing 5-bromo-4-chioro-3-indolyl phosphate and 0.1% sodium azide
7. **Transfer Pipettes**

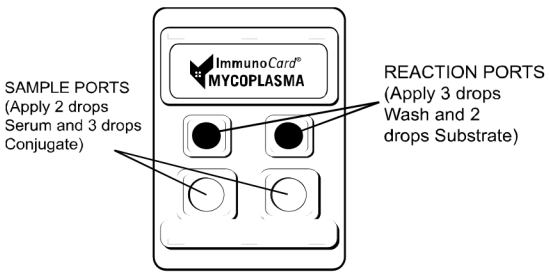
MATERIALS NOT PROVIDED
Timer

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Reagent concentration, incubation times and temperatures (22-25 C) have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications. Once the assay has been started, complete all subsequent steps without interruption.
3. The right reaction (upper) port has been coated with extracted *Mycoplasma* antigens. Handle as a potentially hazardous material.
4. Patient specimens, Positive Control reagent, and used Test Cards may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories".
5. Inspect Test Cards before removing the foil pouch. Do not use Test Cards that have holes in the foil pouch or where the pouch has not been completely sealed. Do not use Test Cards where the desiccant indicator has changed from blue to pink. False-negative reactions may result due to deterioration of the improperly stored Test Cards.
6. The Positive Control contains human sera, which were screened for HBsAg and antibody to HIV-1 and found to be negative. However, no test can offer complete assurance that human blood will not transmit HIV, hepatitis, or other infectious agents.
7. All reagents should be gently mixed and at 22-25 C before use.
8. Do not interchange reagents from different kit lot numbers or use expired reagents.
9. Hold reagent vials and transfer pipettes vertically to insure proper drop size and delivery. Do not allow the tips of the bottle or pipette to touch either the sample or reaction ports.
10. Replace colored caps on correct vials.
11. Substrate Reagent may be light sensitive and should not be exposed to excessive illumination. Substrate Reagent should be colorless. If this reagent exhibits a blue color, it should be discarded.
12. Use only one transfer pipette per control or specimen. Discard after use. Do not attempt to reuse.
13. Disregard any color reactions in the sample (lower) ports. Results are determined by color development in the reaction (upper) ports.
14. Severely lipemic serum, contaminated serum, or serum with excessive debris may restrict movement of Enzyme Conjugate into the sample (lower) ports, potentially producing an invalid result. Noncontaminated serum causing flow problems (invalid results) may be centrifuged and retested.
15. Specimens with obvious microbial contamination or severe hemolysis should not be tested as they may yield unreliable results.
16. Patient samples should not be allowed to dry in the sample application ports. Drying of whole blood or serum onto filter paper inactivates, to varying extents, IgM class antibodies.[12]
    WARNING: Some reagents in this kit contain sodium azide which is a skin irritant. Avoid skin contact with reagents. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.

HAZARD and PRECAUTIONARY STATEMENTS
There are no known hazards associated with this product.

SHELF LIFE AND STORAGE
The expiration date is indicated on the kit label. Store the kit at 2-8 C and return the kit promptly to the refrigerator after each use.

PROCEDURAL NOTES
1. The Test Card format is diagrammed below:



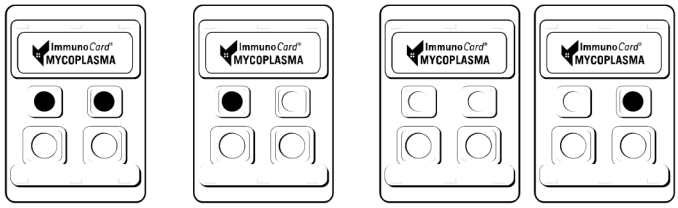
2. Batch processing any number of samples or controls is possible provided that *for each card,* the appropriate steps, sequence of reagent addition, incubation (wait) times and result reading time are maintained. Each procedural step should be completed with each sample before the next step is started.
3. The **CONTROL** (left) side of each card provides a procedural control for each specimen. This tests for proper specimen and reagent flow characteristics as well as reagent performance.

REAGENT PREPARATION
1. Allow all kit components to reach room temperature (22-25 C) before use (requires at least one hour). Gently mix liquid reagents prior to use.
2. All reagents come ready to use (no dilution required)

SPECIMEN COLLECTION AND PREPARATION
Serum specimens obtained from clotted blood should be stored at 2-8 C until tested. The specimen should be tested as soon as possible but may be held up to 72 hours at 2-8 C prior to testing. If testing cannot be performed within this time frame, the specimen should be frozen in a nondefrosting freezer (-20 C or lower) immediately upon receipt. Repeated freezing and thawing of specimens should be avoided.

TEST PROCEDURE
1. Remove the appropriate number of Test Cards from their envelopes. Label with appropriate identification. Use 1 Test Card for each control or sample to be tested.
2. Using a transfer pipette, dispense 2 drops of serum to both lower sample ports.
3. Incubate 2 minutes at 22-25 C. Note: during the 2-minute incubation, specimen must adsorb completely and cover both reaction (upper) ports.
4. Add 3 drops of Enzyme Conjugate to both sample (lower) ports. Incubate 2 minutes at 22-25 C. Enzyme Conjugate should completely absorb during the incubation period.
5. Add 3 drops of Wash Buffer to both reaction (upper) ports. Wait until wash buffer has absorbed completely.
6. Add 2 drops of Substrate Reagent to both reaction (upper) ports. Start a timer for 5 minutes when substrate is added to the first Card. Incubate for 5 minutes at 22-25 C. Visually read results immediately at the end of the incubation period.

INTERPRETATION OF RESULTS



REACTIVE **NONREACTIVE** **INVALID**

READ ONLY UPPER REACTION PORTS TO INTERPRET RESULTS

Reactive Test Result: Visually detectable blue color in **BOTH** reaction ports. Occasionally a reactive test result may show evidence of a gradation of blue color within the reaction port. A reactive result indicates the presence of IgM to *M. pneumoniae.*
Nonreactive Test Result: Visually detectable blue color in **CONTROL** reaction port (upper left) only. The **TEST** reaction port (upper right) should be colorless to faint grey. Occasionally, the **TEST** reaction port (upper right) may show evidence of a hint of blue color in the right or left side of the port, with the rest of the port remaining colorless. This should be considered a nonreactive test result. Nonreactive results indicate either the absence of IgM to *M. pneumoniae*, or levels below the limit of detection for the assay.
Invalid Test Result: No detectable color in **CONTROL** reaction port (upper left). Invalid test results may be due to a reagent/Test Card problem, a procedural error, or restriction of flow of sample and/or Enzyme Conjugate due to severely contaminated, lipemic or debris containing serum. Noncontaminated serum may be centrifuged and retested.

QUALITY CONTROL
This test should be performed per applicable local, state, or federal regulations or accrediting agencies.
The Positive and Negative Controls should be assayed with each new kit lot or each new shipment. Add two drops of Positive Control to both lower Sample ports of a card. Add two drops of Negative Control to both lower Sample ports of a second card. Follow Steps 3 through 6 in the Procedure Section.
1. **Positive Control:** Must yield visually detectable blue color in both reaction (upper) ports.
2. **Negative Control:** Must yield visually detectable blue color in **CONTROL** (upper left) reaction port only. The **TEST** (upper right) reaction port should be colorless.

The **Procedural Control** present in the upper left port of each Test Card tests the individual specimen for proper flow and reagent performance. Failure of the **Procedural Control** to yield a blue color with any specimen or control reagent indicates an invalid test result and the test should be repeated.

At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, or leakage.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

It is suggested that the results of each quality control check be recorded in an appropriate log book to maintain high quality testing records. If the expected reactions are not observed and the reagents are still within their expiration date, please contact Meridian Bioscience's Technical Support Services at 513-271-3700 or 800-343-3858 (for US only) or contact your Country/Local Distributor.

EXPECTED VALUES
The Immuno*Card* Mycoplasma test was evaluated at four hospitals throughout the midwest. In addition, a reference lab tested specimens from throughout the country. Of the 160 prospective specimens tested at the hospital sites, 26 (16%) were positive by the Immuno*Card* Mycoplasma test. The reference laboratory reported 85/352 (24%) Immuno*Card* Mycoplasma positive specimens. These results were consistent with results obtained using other IgM tests for *M. pneumoniae,* as well as published findings for the prevalence of IgM to *M. pneumoniae*.[1, 2, 13, 14] When a group of blood bank sera was tested for IgM to *M. pneumoniae* using the Immuno*Card* Mycoplasma and a reference EIA, a prevalence of 12.7% and 16% was found by each method, respectively.

LIMITATIONS OF THE PROCEDURE
1. Immuno*Card* Mycoplasma test results should be used in conjunction with information available from the patient clinical evaluation and other available diagnostic procedures.
2. Samples obtained too early during infection may not contain detectable levels of IgM antibody. If a *M. pneumoniae* infection is suspected, a second sample should be obtained in 7-14 days and tested.
3. Significance of test results of immunosuppressed patients may be difficult to interpret.
4. Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.
5. Specific IgM antibodies to *M. pneumoniae* are usually detected in patients with a recent primary infection. However, they may be found in patients with reactivated or secondary infections and are sometimes found in patients with no other detectable evidence of recent infection.[7, 11] In addition, IgM to *M. pneumoniae* has been shown to persist for extended periods (2-12 months) in some patients.[11]
6. False negative results due to competition by high levels of IgG, while theoretically possible, have not been observed.

SPECIFIC PERFORMANCE CHARACTERISTICS
The Immuno*Card* Mycoplasma test was evaluated using sera at three hospitals and one reference laboratory. Immuno*Card* Mycoplasma results were compared with a microwell EIA for IgM to *M. pneumoniae.* Discrepant results were resolved by IFA, latex and complement fixation testing.

Immuno*Card*	Reference EIA			Resolved		
	Reactive	Nonreactive	Retest	Reactive	Nonreactive	Retest
Reactive	69	45	16	88	29	13
Nonreactive	27	245	12	12	268	4

Relative Sensitivity 88% ± 6%[1]
Relative Specificity 90% ± 3%
Relative Agreement 90% ± 3%
[1] ± values calculated as 95% confidence intervals using the normal method.

Forty-five Immuno*Card* Mycoplasma reactive specimens, which were nonreactive by the reference EIA had 14 reactive, 26nonreactive, and five unresolved results. Sixteen sera with reactive Immuno*Card* and indeterminate reference EIA results had five reactive, three nonreactive, and eight unresolved results.

Twenty-seven specimens with Immuno*Card* nonreactive, reference EIA reactive results had 11 reactive, 14 nonreactive, and two unresolved results. Finally, 12 Immuno*Card* nonreactive, reference EIA indeterminate specimens had one reactive, nine nonreactive, and two unresolved results. No Immuno*Card* Mycoplasma invalid test results were obtained during clinical trials compared to 28/414 or a 6.8% retest rate for the reference EIA method.

Two of the clinical trial sites (one hospital and a reference lab) performed complement fixation titration for antibody to *M. pneumoniae.* CF results were grouped as nonreactive (< 1/8), low (1/8-1/32), and reactive (≥ 1/64). Immuno*Card* Mycoplasma results were compared with CF titers in the table below.

	CF Titer		
Immuno*Card*	< 8	8-32	≥ 64
Reactive	29	25	28
Nonreactive	97	56	4

The Immuno*Card* Mycoplasma test correctly identified 28/32 (88%) of the high titer (≥ 64) CF specimens. The Immuno*Card* test was reactive with 25 (31%) of the low titer CF (8-32) specimens. Twenty-four (96%) of these were confirmed as IgM reactive by other methods. Finally, 25/29 (86%) CF nonreactive specimens were found reactive by Immuno*Card* Mycoplasma and were also reactive by either EIA or IFA.

ANALYTICAL SPECIFICITY
The specificity of the Immuno*Card* Mycoplasma test was evaluated on retrospective specimens from patients with positive culture or with serological evidence for other atypical pneumonias, as well as viral, bacterial, and fungal pneumonias. Specimens positive for rheumatoid factor, anti-nuclear antibody and lupus were also tested. One of four lupus specimens was Immuno*Card* Mycoplasma reactive. No cross-reactions were observed with the other classes of sera listed below. Values in parentheses indicate the number of sera tested.

Histoplasma (5)
Parainfluenza 3 (1)
Influenza B (1)
Adenovirus (3)
Cytomegalovirus (6)
Legionella (7)
Antinuclear Antibody (20)
Coccidioides (10)
Influenza A (13)
Respiratory Syncytial Virus (1)
Epstein Barr Virus (14)
Chlamydia (9)
Rheumatoid Factor (10)

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INTERNATIONAL SYMBOL USAGE
You may see one or more of these symbols on the labeling/packaging of this product:
Key guide to symbols (Guida ai simboli, Guide des symbol es, Guia de simbolos, Zeichenerklärung)

	Use By / Utilizzare entro / Utiliser jusque / Fecha de caducidad / Verwendbar bis	CONTROL +	Positive control / Controllo positivo / Contrôle positif / Control positivo / Positive Kontrolle
LOT	Batch Code / Codice del lotto / Code du lot / Código de lote / chargenbezeichnung	CONTROL −	Negative control / Controllo negativo / Contrôle négatif / Control negativo / Negative Kontrolle
IVD	In vitro diagnostic medical device / Dispositivo medico-diagnostico in vitro / Dispositif médical de diagnostic in vitro / Dispositivo médico para diagnóstico in vitro / In-Vitro-Diagnostikum	EC REP	Authorized representative in the European Community / Rappresentante Autorizzato nella Comunitá Europea / Mandatiare dans la Communauté européanne / Representante autorizado en la Comunidad Europea / Bevollmächtigter in der Europäischen Gemeinschaft
CE	This product fulfils the requirements of Directive 98/79/EC on in vitro diagnostic medical devices / Questo prodotto soddisfa i requisiti della Direttiva 98/79/CE sui dispositivi medico-diagnostici in vitro / Ce produit répond aux exigences de la Directive 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro / Este producto cumple con las exigencias de la Directiva 98/79/CE sobre los productos sanitarios para diagnóstico in vitro / Dieses Produkt entspricht den Anforderungen der Richtlinie über In Vitro Diagnostica 98/79/EG.	SMP PREP DIL SPE	Sample Preparation Apparatus containing Sample Diluent / Dispositivo per la preparazione del campione contenente il diluente del campione / Système pour la préparation de l'échantillon, diluant inclus / Aparato para Preparación de Muestra con Diluyente de Muestra / System zur Probenvorbereitung, in dem sich Probenverdünngspuffer befindet
			CAUTION: Risk of Danger / ATTENZIONE: Pericolo / AVERTISSEMENT: Risques de danger / Precaución: Peligroso / WARNUNG: Risikogefahr
REF	Catalogue number / Numero di catalogo / Référence du catalogue / Numero de catálogo / Bestellnummer		Do not freeze / Non congelare / Ne pas congeler / No congelar / Nicht Eingrieren
	Consult Instructions for Use / Consultare le istruzioni per l'uso / Consulter les instructions d'utilisation / Consulte las instrucciones de uso / Gebrauchsanweisung beachten	BUF RXN	Reaction Buffer / Tampone di reazione / Solution de réaction tamponnée / Tampón de Reacción / Reaktionspuffer
	Manufacturer / Fabbricanto / Fabricant / Fabricante / Hersteller		For IVD Performance Evaluation Only / Soltanto per valutazione delle prestazioni / Réactifs IVD reservés á l'évaluation des performances / Sólo para evaluación del funcionamiento / Nur zur IVD Leistungsbewertung
Σ	Contains sufficient for <n> tests / Contenuto sufficiente per "n" saggi / Contenu suffisant pour "n" test / Contenido suficiente para <n> ensayos / Inhalt ausreichend für <n> Prüfungen	SOLN STOP	Stopping Solution / Soluzione di Stop / Solution d'arrêt / Solución de parada / Stopplösung
	Temperature limitaion / Limiti di temperatura / Limites de température / Limite de temmperatura / Temperaturbegrenzung	CONJ ENZ	Enzyme Conjugate / Coniugato enzimatico / Conjugué enzymatique / Conjugado enzimático / enzymkonjugat
SN	Serial number / Numero di serie / Numéro de série / Número de serie / Seriennummer	CONTROL	Assay Control / Controllo del test / Test de contrôle / Control de Ensayo / Kontrolltest
TEST	Test Device / Dispositivo test / Dispositif de test / Dispositivo de Prueba / testgarät	REAG	Reagent / Reagente / Réactifs / Reactivos / Reagenzien
	Date of manufacture / Data di fabbricazione / Date de fabrication / Fecha de fabricación / Herstellungsdatum	BUF WASH	Wash Buffer / Soluzione di lavaggio / Solution de lavage / Tampón de lavado / Waschpuffer
BUF	Buffer / Soluzione tampone / Solution tamponnée / Tampón / Puffer		Warning / Avvertenze / Mise En Garde / Advertencia / Warnhinweise
CONJ	Conjugate / Coniugato / Conjugué / Conjugado / Konjugat	DIL SPE	Specimen Diluent (or Sample Diluent) / Diluente del Campione / Diluant échantillons / Diluyente de muestra / Probenverdünnungspuffer
SUBS	Substrate / Substrato / Substrat / Substrato / Substrat	BUF WASH 20X	Wash Buffer Concentration 20X / Soluzione dil lavaggio 20X / Solution de lavage concentrée 20X / Solución tampón de lavado 20X / 20fach konzentriertes Waschkonzentrat
Rx Only	Prescription Use Only / Per l'uso su prescrizione medica / Uniquement sur prescription / Solo Para Uso Por Receta / verschreibungspflichtig	DET REAG	Detection Reagent / Reagente Diretto / Réactif de Detection / Reactivo de Detección / Nachweis Reagenz
	Do not use if package is damaged / Non utilizzare se la confezione è danneggiata / ne pas utiliser si le paquet est endommagé / No use si el paquete esta dañado / Nicht verwenden, wenn die Verpackung beschädigt ist	TUBE	Empty Tube / Provetta vuota / Tube vide / Tubo vacio / Leeres Gefäß

For technical assistance, call Technical Support Services at 800-343-3858 between the hours of 8AM and 6PM, USA Eastern Standard Time. To place an order, call Customer Service Department at 800-543-1980.