



AccuDiag™ Mycoplasma pneumoniae IgG ELISA Kit

REF 8042

PIC ID8042ZY1

IVD See External Label 2°C 8°C 96 Tests

SIGNIFICANCE AND SUMMARY

Mycoplasma pneumoniae is among the smallest of free-living organisms. In humans, it is usually found in the throat and lungs and is susceptible to antimicrobial agents that inhibit protein synthesis.¹ The spread of mycoplasma infections depends on close and prolonged contact between people, as it is transmitted by means of droplet nuclei from the respiratory tract.² Mycoplasma infection may be asymptomatic, or may produce upper respiratory tract disease, or atypical pneumonia. The pneumonia is difficult to differentiate from viral diseases by clinical means alone.^{1,2} Laboratory tests such as isolation, Complement Fixation serology and ELISA serology are helpful as aids in the diagnosis of Mycoplasma pneumoniae infection.^{1,2,3,4}

ASSAY PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials, (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, Tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is indicative of the presence of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.^{5,6,7,8} The % agreement positive, % agreement negative, and reproducibility of enzyme-linked immunoassays can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays.^{9,10,11}

SPECIMEN COLLECTION & PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, nonhemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.¹³ Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2 and 8°C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. If paired sera are to be collected, acute samples should be collected as soon as possible after onset of symptoms and not later than seven days after onset. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate on the same plate to test for a significant rise in antibody. If the first specimen is obtained too late during the course of the infection, a significant rise may not be detectable.

Mycoplasma pneumoniae IgG ELISA	
Principle	Indirect ELISA
Detection	Qualitative/Semi-Quantitative
Sample	10 µL serum/plasma
Incubation Time	60 minutes
Sensitivity	99.3%
Specificity	92.1%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. AccuDiag™ Mycoplasma IgG Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the semi-quantitative or qualitative determination of IgG antibodies in human serum to Mycoplasma pneumoniae for the determination of immunological experience. The Diagnostic Automation Inc. Mycoplasma IgG ELISA kit may be used to evaluate paired sera for the presence of seroconversions and a significant increase in specific IgG as an aid in the diagnosis of Mycoplasma pneumoniae infection in the adult population. **For *in vitro* diagnostic use. High complexity test.**



- The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18A, 1990).

REAGENTS

Materials provided with the kit

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

- Mycoplasma (FH strain, grown in PPLO broth, washed, detergent treated, and sonicated) antigen coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
- Serum Diluent Type 1: Ready for use. Contains ProClin® (0.1%) as a preservative (96T: one bottle, 30 mL)
- Cutoff Calibrator (Calibrator): human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Cutoff Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature. (96T: one vial, 0.4 mL)*
- High Positive Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the upper dynamic range of the assay. (96T: one vial, 0.4 mL)*
- Low Positive Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with range printed on vial label. The Low Positive Control is utilized to control the assay near the cutoff of the assay. (96T: one vial, 0.4 mL)*
- Negative Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with range printed on vial label. The negative control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL)*
- Horse-radish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, containing ProClin® (0.1%) as a preservative. (96T: one bottle, 16 mL)
- Chromogen/Substrate Solution Type 1: Tetramethylbenzidine (TMB), ready to use. (96T: one bottle, 15 mL)
- Wash Buffer Type 1 (20X concentrate): dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative. (96T: one bottle, 50 mL)
- Stop Solution: Contains a 1N H₂SO₄ solution, ready to use. (96T: one bottle, 15 mL)

Note: serum vials may contain excess volume.

Materials required but not provided

- Graduated cylinder (100 mL)
- Flask (1 L)
- Timer – 0 to 60 minutes
- Micropipettes capable of accurately delivering 10-200 µL volumes (less than 3% CV).
- Deionized or distilled water.
- Paper towels.
- Wash bottle, semi-automated or automated wash equipment.
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.
- Test tubes for serum dilution.
- Disposal basin and disinfectant (e.g., 0.5% sodium hypochlorite)

Note: Use only clean, dry glassware.

REAGENT PREPARATION

- All reagents must be removed from refrigeration and allowed to come to room temperature before use (21 to 25°C). Return all reagents to refrigerator promptly after use.
- All samples and controls should be vortexed before use.
- Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H₂O. Mix well.

ASSAY PROCEDURE

Note: To evaluate paired sera, both serum samples must be tested in duplicate and run on the same plate. It is recommended that the serum pairs be run in adjacent wells.

- Place the desired number of strips into a microwell frame. Allow six (6) Control/Cutoff Calibrator determinations (one Negative Control, three Cutoff Calibrators, one High Positive Control, and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

RB = Reagent Blank – Well without serum addition run with all reagents.

Utilized to blank reader.

NC = Negative Control

Cal = Calibrator

HPC = High Positive Control

LPC = Low Positive Control

- Dilute test sera, Cutoff Calibrator, High, Low and Negative Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. (For manual dilutions, it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
- To individual wells, add 100 µL of the appropriate diluted Cutoff Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
- Incubate each well at room temperature (21 - 25°C) for 25 minutes +/- 5 minutes.
- Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment, add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four (4) times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.



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I M M U N O D I A G N O S T I C S

IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results, the use of semiautomated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Please contact Diagnostic Automation Inc. with any questions regarding appropriate wash equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

- Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
- Incubate each well **25 minutes +/- 5 minutes** at room temperature (21 – 25°C).
- Repeat Wash as described in Step 5.
- Add 100 µL Chromogen/Substrate Solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
- Incubate each well **10 - 15 minutes** at room temperature (21 to 25°C).
- Stop reaction by addition of 100 µL of Stop Solution (1N H₂SO₄) following the same order of Chromogen/Substrate Solution addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
- The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is > 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

- Cutoff Calibrator Value - Calculate the mean value for the Cutoff Calibrator from the three Cutoff Calibrator determinations. If any of the three Cutoff Calibrator values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
- Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined for each lot of kits. The Correction Factor is printed on the Cutoff Calibrator vial.
- Cutoff O.D. Value - The Cutoff O.D. Value for each assay is determined by multiplying the Correction Factor by the mean Cutoff Calibrator Value determined in step 1.
- ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. value by the Cutoff O.D. Value determined in step 3.

Example:

O.D.'s obtained for Calibrator	= 0.38, 0.42
Mean O.D for Calibrator	= 0.40
Correction Factor	= 0.50
Cutoff Calibrator Value	= 0.50 x 0.40 = 0.20
O.D. obtained for patient sera	= 0.60
ISR Value	= 0.60/0.20 = 3.00

- The maximum linearity of the assay is an ISR of 3.18, therefore ISR Values of >3.18 should be reported as greater than 3.18.

ANALYSIS

- The patients' ISR (Immune Status Ratio) is interpreted and reported as follows:

ISR	Results	Interpretation
≤ 0.90	Negative	Suggests no prior immunological exposure. No detectable to Mycoplasma pneumoniae; result does not rule out recent exposure and collection of test sample prior to development of IgG. Culture is recommended for determining current infection.
0.91 – 1.09	Equivocal	Immunological exposure cannot be assessed. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken
≥ 1.10	Positive	Indicates presence of detectable IgG antibody Detectable IgG. Suggests immunological exposure to mycoplasma Mycoplasma pneumoniae.

- To determine the cutoff of the assay, twenty-eight negative sera were assayed by the Diagnostic Automation Inc. Mycoplasma IgG ELISA assay. The negativity and positivity of specimens used to determine the cut-off for the assay were determined by another ELISA method. The mean and standard deviation of the optical density readings for the sera was 0.19 and 0.077, respectively. The positive threshold for the assay was determined by adding the mean and two standard deviations (0.19 + 2 (0.077) = 0.34). A positive serum was titrated to give a constant ratio of the threshold value to obtain a Cutoff Calibrator serum. On all subsequent assays, this serum was run and the assay was calibrated by multiplying the O.D. Value for the Cutoff Calibrator by the ratio to the cutoff to obtain the Cutoff O.D. This value was then divided into the O.D. for the patient sera to obtain an Immune Status Ratio (ISR). By definition, the cutoff ISR is equal to 1.00. To account for inherent variation in immunoassay, values of 0.91-1.09 are considered equivocal. Therefore, values < 0.90 are considered negative and values >1.10 are considered positive.
- The following is a recommended method for reporting the results obtained; "The following results were obtained with the Diagnostic Automation Inc. Mycoplasma IgG ELISA. Values obtained with different methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer."
- To evaluate paired sera for a significant change in antibody level, both samples must be tested in duplicate in the same assay. The mean ISR for the convalescent sample must be > 1.10 to evaluate the paired sera for a significant rise in antibody level.^{15,16}
- Additional Quality Control for Paired Sera: (See NOTE under General Procedure). As a check for acceptable reproducibility of both the acute sera (tested in duplicate) and the convalescent sera (tested in duplicate), the following criteria must be met for valid results:

Acute 1 ISR = 0.8 to 1.2 Convalescent 1 ISR = 0.8 to 1.2

Acute 2 ISR Convalescent 2 ISR

- Compare the ISR of the pairs by calculating as follows:

Mean ISR (convalescent sample) - Mean ISR (acute sample)

Mean ISR (first sample) X 100 = % RISE IN ISR LEVEL

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% RISE IN ISR	INTERPRETATION
< 46.0%	No significant change in antibody level. No evidence of recent infection. If active disease is still suspected, a third sample should be collected and tested in the same assay as the first sample to look for a significant rise in antibody level.
≥ 46.0%	Statistically significant change in antibody level detected. This is indicative of acquiring an active Mycoplasma pneumoniae infection in the past 1 to 6 months

- When evaluating paired sera, the acute serum must be < 2.18, due to the maximum linearity of the assay.
- When evaluating paired sera, it should be determined if samples with high absorbance values are within linearity specifications of the spectrophotometer. Read the Operator's Manual or contact the instrument's manufacturer to obtain the established linearity specifications of your spectrophotometer.

QUALITY CONTROL

- For the assay to be considered valid the following conditions must be met:
- Calibrator and controls must be run with each test run.
 - Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
 - Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
 - Each Cutoff Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).
 - High Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).
 - The ISR (Immune Status Ratio) Values for the High, Low, and Negative Controls should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
 - Additional controls may be tested according to guideline, or requirements of local, state, and/or federal regulations or accrediting organizations.
 - Refer to NCCLS C24-A for guidance on appropriate Quality Control practices.
 - If the above criteria are not met on repeat testing, contact Diagnostic Automation Inc. Technical Service.

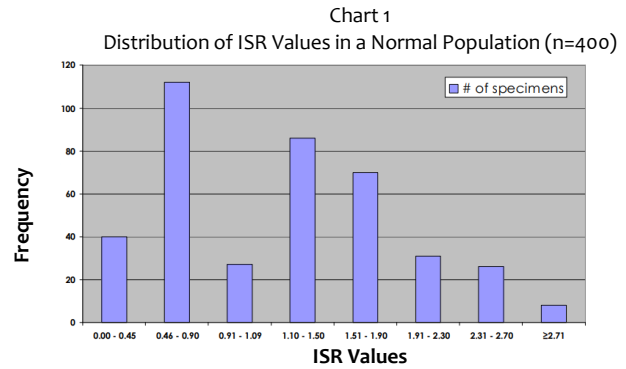
EXPECTED RANGES OF VALUES

Currently, culture of Mycoplasma pneumoniae and a four-fold rise in antibody levels are the laboratory tests used as an aid in the diagnosis of Mycoplasma pneumoniae infection. Culture is not a sensitive method as only ~58% of patients with a four-fold increase in antibody level by Complement Fixation (CF) will be culture positive.¹² Conversely, four-fold increases in antibody level by CF are seen in ~53% of culture positive patients.¹² Many Mycoplasma pneumoniae infections are asymptomatic and reinfection can occur. The incidence of Mycoplasma pneumoniae IgG antibodies in the general population is high.³

PREVALENCE

A population of 400 serum samples collected from a healthy population with random gender, age (18 – 50), race and geographic location were tested on the Diagnostic Automation Inc. Mycoplasma IgG ELISA assay. The Diagnostic Automation Inc. Mycoplasma IgG ELISA demonstrated a 55.25 % positivity rate

for this group of samples. The distribution of ISR values from this study is presented in the following chart.



PERFORMANCE CHARACTERISTICS

% AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE Evaluation of Mycoplasma IgG ELISA % Agreement Positive and % Agreement Negative Relative to IFA

The Diagnostic Automation Inc. Mycoplasma IgG ELISA was evaluated by masked testing of 200 serum versus a commercially available IFA. Table 1 illustrates the % agreement positive and % agreement negative relative to a commercially available IFA assay.

Table 1
Diagnostic Automation Inc. Mycoplasma IgG ELISA
% Agreement Positive and % Agreement Negative

Diagnostic Automation Inc. Mycoplasma IgG ELISA					
Mycoplasma IFA (1:32)	+	eq	-	Total	
+	98	9	21	128	
-	6	4	62	72	
Total	104	13	83	200	

95% Confidence Interval

% Agreement Positive = 98/119 = 82.4% 75.5% - 89.2%
 % Agreement Negative = 62/68 = 91.2% 84.4% - 97.9%
 % Agreement = 160/187 = 85.6% 80.5% - 90.6%

Equivocals were not included in the above calculations.

The 95% Confidence Intervals were calculated using the normal method.

Evaluation of Mycoplasma Igg ELISA % Agreement Positive and % Agreement Negative Relative to an Alternate ELISA

The Diagnostic Automation Inc. Mycoplasma IgG ELISA was evaluated by masked testing of 478 sera versus a commercially available alternate ELISA. Table 2 illustrates the % agreement positive and % agreement negative relative to the alternate ELISA assay.

Table 2
Diagnostic Automation Inc. Mycoplasma IgG ELISA
% Agreement Positive and % Agreement Negative

Diagnostic Automation Inc. Mycoplasma IgG ELISA					
Alternate ELISA	+	eq	-	Total	
+	169	3	10	182	



	eq	10	7	0	17
	-	86	26	167	279
	Total	265	36	177	478

95% Confidence Interval

% Agreement Positive = 169/179 = 94.4% 91.0% - 97.8%
 % Agreement Negative = 167/253 = 66.0% 60.2% - 71.8%
 % Agreement = 343/478 = 77.8% 73.9% - 81.7%

Equivocals were not included in the above calculations.

The 95% Confidence Intervals were calculated using the normal method.

Evaluation of Mycoplasma Igg ELISA % Agreement Positive and % Agreement Negative Relative to a Second Alternate ELISA

The Diagnostic Automation Inc. Mycoplasma IgG ELISA was evaluated by masked testing of 200 sera from a healthy population with random gender, age (18 – 50), race and geographic location versus a second commercially available alternate ELISA. Testing was conducted at an R&D laboratory at a commercial company located in Maryland. Table 3 illustrates the % agreement positive and % agreement negative relative to the second alternate ELISA assay.

Table 3
Diagnostic Automation Inc. Mycoplasma IgG ELISA
% Agreement Positive and % Agreement Negative

Diagnostic Automation Inc. Mycoplasma IgG ELISA						
Alternate ELISA		+	eq	-	Total	
		+	146	3	0	149
		eq	1	11	3	15
		-	0	1	35	36
		Total	147	15	38	200

95% Confidence Interval

% Agreement Positive = 146/146 = 100.0% 97.5% - 100%
 % Agreement Negative = 35/35 = 100.0% 90.0% - 100%
 % Agreement = 192/192 = 100.0% 98.1% - 100%

Equivocals were not included in the above calculations.

The 95% Confidence Intervals were calculated using the normal method.

PRECISION

The precision of the Diagnostic Automation Inc. Mycoplasma IgG ELISA was determined by testing six different sera ten times each on three separate days at two different sites. Both sites were affiliated with the manufacturer of the kit. The intra- and inter-assay precision at each site is shown in Tables 4 and 5. The inter-site precision is shown in Table 6. With appropriate technique the user should obtain precision of < 15% CV.

Table 4

Mycoplasma IgG ELISA Intra- and Inter-Assay Precision Study 1

Sera#	Assay 1 (n=10)				Assay 2 (n=10)				Assay 3 (n=10)				Inter Assay (n=30)			
	X	SD	CV%		X	SD	CV%		X	SD	CV%		X	SD	CV%	
1	2.06	0.19	9.11	2.24	0.16	7.33	2.12	0.14	6.76	2.14	0.18	8.33				
2	2.05	0.07	3.17	2.30	0.17	7.30	2.08	0.13	6.20	2.14	0.17	7.73				
3	1.37	0.10	7.01	1.51	0.09	6.17	1.35	0.07	5.33	1.41	0.11	7.92				
4	1.08	0.11	10.60	1.28	0.13	10.01	1.06	0.09	8.20	1.14	0.15	12.86				
5	0.27	0.02	8.42	0.30	0.02	5.38	0.26	0.01	4.29	0.28	0.02	8.94				
6	0.38	0.03	8.07	0.42	0.03	6.32	0.35	0.04	11.11	0.38	0.04	11.34				

Table 5

Mycoplasma IgG ELISA Intra- and Inter-Assay Precision Study 2

Sera#	Assay 1 (n=10)				Assay 2 (n=10)				Assay 3 (n=10)				Inter Assay (n=30)			
	X	SD	CV%		X	SD	CV%		X	SD	CV%		X	SD	CV%	
1	2.07	0.13	6.46	2.42	0.13	5.55	2.03	0.10	5.02	2.17	0.21	9.86				
2	2.09	0.17	8.03	2.45	0.08	3.17	2.03	0.06	3.06	2.19	0.22	9.81				
3	1.46	0.09	6.05	1.82	0.13	7.00	1.54	0.08	5.49	1.60	0.19	11.59				
4	1.14	0.09	8.36	1.26	0.13	10.01	1.06	0.09	8.73	1.15	0.13	11.38				
5	0.30	0.02	7.45	0.34	0.04	10.31	0.31	0.04	12.01	0.32	0.04	11.33				
6	0.42	0.04	10.06	0.46	0.05	11.15	0.38	0.04	10.19	0.42	0.05	12.47				

Table 6

Diagnostic Automation Inc. Mycoplasma IgG ELISA Inter-Site Precision Study

Sera #	Inter-Assay				n
	X	SD	CV		
1	2.16	0.20	9.10		60
2	2.17	0.19	8.86		60
3	1.51	0.18	11.98		60
4	1.15	0.14	12.04		60
5	0.30	0.04	12.63		60
6	0.40	0.05	12.78		60

X = Mean

SD = Standard Deviation

CV = Coefficient of Variation = SD/X x 100

The methods in NCCLS EP5 were utilized for precision parameters.

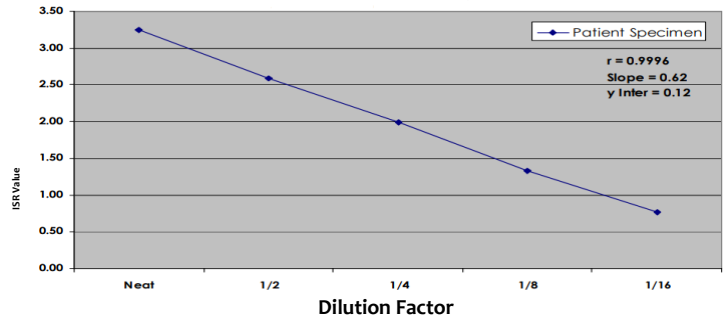
LINEARITY

Simulated Paired Sera Evaluation

To evaluate the linearity of the assay 22 positive sera were serially two-fold diluted and run on the assay. The ISR values were compared to log₂ of dilution by standard linear regression. The r values were all > 0.990. The data indicate that the antibody can be semi-quantitated by using a single serum dilution. The detection of a significant antibody increase may be made only by an evaluation of paired specimens, acute and convalescent. To validate the % agreement positive of the paired sera procedure, the percent rise in ISR value was calculated for 42 pairs that had a four-fold dilution where the acute sera had a value of less than 2.18. All 42 pairs demonstrated a > 46% rise in ISR value, showing a significant rise in antibody. Therefore, the paired sera procedure demonstrated 100% agreement positive in being able to detect a four-fold increase in antibody level when the acute sera has a value of < 2.18.

Chart 2 illustrates the linearity of a representative serum. The ISR values were compared to log₂ of dilution by standard linear regression.

Chart 2
Linearity of Mycoplasma IgG ELISA



REPRODUCIBILITY STUDY

200 different sera with various levels of activity were assayed at two different testing sites. Both sites were R&D laboratories at commercial companies located in Maryland and New York. Excluding equivocals, two determinations varied from its expected result (negative result for a positive specimen) giving a percent agreement of expected results between the two sites of 99.5% (398/400).

LIMITATIONS OF THE ASSAY

- The state of present-day technology does not provide a recommended reference standard. Because of the current inconsistencies in various test methodologies, physicians and laboratories must rely on a combination of test methods and results, and clinical symptoms when making a diagnosis of Mycoplasma pneumoniae infection. The Diagnostic Automation Inc. Mycoplasma IgG ELISA test is not intended to replace culture, and should not be used as the sole basis for diagnosis.



2. Kit procedures or practices outside those in this package insert may yield questionable results.
3. Icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and should be avoided.
4. False positive results may occur with sera from patients with Ureaplasma, Mycoplasma hominis, Mycoplasma genitalium, pancreatitis, bacterial meningitis and other acute inflammatory disease. Cross-reactivity of this assay with antibodies to the above disease states has not been determined. Epidemiology of case, symptoms and other laboratory tests can help in differentiating these conditions from Mycoplasma pneumoniae infection.
5. A negative antibody result does not rule out Mycoplasma pneumoniae infection. False negative results may occur when samples are drawn too early after onset. Production of detectable antibody levels may be delayed. Some patients may never generate detectable antibody levels. Patients with symptoms suggestive of Mycoplasma pneumoniae infection with negative test results should be retested in 4 - 6 weeks using paired sera analysis.
6. Mycoplasma pneumoniae infection can have a long incubation period, thus elevated antibody titers in the acute specimen are common, and reinfection may occur, therefore sero-conversions (negative to positive) are unusual.
7. A positive single serum result only indicates prior exposure to Mycoplasma pneumoniae. The antibody level in a single specimen does not have significance for disease severity. The presence or absence of antibody cannot be used to determine the success or failure of antibiotic therapy.
8. Screening of the general population should not be performed. Testing should only be performed when clinical symptoms are present or exposure is suspected.
9. The results of ELISA immunoassays performed on serum from immunosuppressed patients must be interpreted with caution.
10. Assay performance characteristics have not been established with specimens from patients with documented mycoplasma infection, nor with matrices other than serum.
11. The prevalence of the analyte will affect the assay's predictive value.

STORAGE CONDITIONS

1. Store unopened kit between 2 and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2 and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2 and 8°C.
3. Store HRP Conjugate between 2 and 8°C.
4. Store the Cutoff Calibrator, High Positive Control, Low Positive Control and Negative Control between 2 and 8°C.
5. Store Serum Diluent and 20X Wash Buffer between 2 and 8°C.
6. Store the Chromogen/Substrate Solution between 2 and 8°C.
7. Store 1X (diluted) Wash Buffer at room temperature (21 to 25°C) for up to 5 days, or 1 week between 2 and 8°C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. The human serum components used in the preparation of the Controls and Cutoff Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to Human Immunodeficiency Virus 1 & 2 (HIV 1&2) and Hepatitis C (HCV) as well as Hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, Hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.14
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution, Stop Solution and Wash Buffer. Serum Diluent supplied with IgG kits can be used only with other IgG kits and Serum Diluent supplied with IgM kits can only be used with other IgM kits. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21 - 25°C) before running assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
7. Before opening Control and Cutoff Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite solutions (bleach) to avoid formation of poison gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Mycoplasma in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.



Diagnostic Automation/Cortez Diagnostics, Inc.

I M M U N O D I A G N O S T I C S



WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.


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MANUFACTURER AND BRAND DETAILS

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Quality
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CERTIFIED

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Date Adopted	2023-11
Brand Name	AccuDiag™
REF 8042	AccuDiag™ - Mycoplasma pneumoniae IgG ELISA
PIC	ID8042ZY1
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