Human Myeloperoxidase IgG ELISA Kit

Cat. No.:DEIA1698
Pkg.Size:96T

Intended use

The Myeloperoxidase IgG ELISA test system is intended for the qualitative and semi-quantitative detection of IgG-class antibody to myeloperoxidase in human serum. The test system is intended to be used as an aid in the diagnosis of various autoimmune vasculitic disorders characterized by elevated levels of antineutrophil cytoplasmic antibodies (ANCA). MPO-ANCA may be associated with autoimmune disorders such as Wegener's granulamotosis, ICGN, MPA and PRS.

General Description

Anti-neutrophil cytoplasmic antibody (ANCA) was initially described by Davies, et al. in 1982. Since this initial discovery, ANCA has been found to be associated with a number of Systemic Vasculitides (SV). Scientists now recognized ANCA to include two primary specificities: C-ANCA directed against Proteinase-3 (PR-3), and P-ANCA directed against Myeloperoxidase (MPO). Testing for both P-ANCA and C-ANCA is highly recommended in the laboratory workup of patients who present with clinical features suggestive of SV. The clinical syndromes most frequently associated with ANCA are as follows:
Wegener's granulomatosis
Polyarteritis
"Overlap" Vasculitis
Idiopathic Crescentic Glomerulonephritis (ICGN)
Kawasaki Disease
Although the initial identification of C-ANCA and P-ANCA was based on the indirect immunofluorescence procedures, further identification and purification of PR-3 and MPO has resulted in the development of enzyme immunoassays (ELISA) for both PR-3 and MPO.

Principle Of The Test

The Myeloperoxidase IgG ELISA test system is designed to detect IgG class antibodies to MPO in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with MPO antigen. The test procedure involves three incubation steps:
1. Test sera (properly diluted) are incubate antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove un-reacted conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

Reagents And Materials Provided

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. All reactive reagents contain sodium azide as a preservative at a concentration of <0.1% (w/v).
1. Plate. 96 wells configured in twelve 1x8 well strips coated with inactivated Myeloperoxidase antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.

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2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One 15 mL vial with a white cap.
3. Positive Control (Human Serum). One 0.35 mL vial with a red cap.
4. Calibrator (Human Serum). One 0.5 mL vial with a blue cap.
5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
6. SAVe diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. (pH 7.2 ±/- 0.2). ready to use.
Note: Shake Well Before Use.
Note: The diluent will change color in the presence of serum.
7. TMB: One 15 mL amber capped bottle containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to se. Contains DMSO < 15% (w).
8. Stop Solution: One 15 mL bottle (red cap) containing 1M H2S04, 0.7M HCI. Ready to use.
9. Wash buffer concentrate (10X): dilute 1 part concentrate ± 9 parts deionized or distilled water. One 100 mL bottle clear capped containing a 10X concentrated phosphate-buffered-saline and Tweeen-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ±/- 0.2.
The following components are not kit lot number dependent and may be used interchangeably with ELISA assays, TMB, Stop Solution, and Wash Buffer.
Note: Kit also contains:
1. Components list containing lot specific information is inside the kit box.
2. Package Insert providing instructions for use.

Materials Required But Not Supplied

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200 µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or plate washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant, (Example: 10% household bleach, 0.5% sodium hypochlorite).

Storage

1. Store the unopened kit at 2-8°C.
2. Coated microwell strips: Store between 2°C and 8°C. Immediately reseal extra strips with desiccant and returned to proper storage. After opening the envelope the strips are stable for 60 days the indicator strips on the desiccant pouch remains blue, demonstrating a proper seal.
3. Conjugate: Store between 2°C and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2°C and 8°C.
5. TMB substrate solution: Store at 2-8°C.
6. Wash buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25°C) for up to 7 days or for 30 days between 2-8°C.
7. SAVe Diluent: Store between 2° and 8°C.

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8. Stop Solution: Store at 2 and 25°C.

Specimen Collection And Handling

1. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

Assay Steps

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and returned to storage between 2° and 8°C.
3. Prepare a 1:21 dilution (e.g: 10 µL of serum ± 200 µL of SAVe Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. When combined with the specimen, the SAVe Diluent will undergo a color change as confirmation.
4. To individual wells, add 100 µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100 µL of SAVe Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25 ℃) for 25 ±/- 5 minutes.
7. Wash the microwell strips 5 times.

Manual Wash procedure

a. Vigorously shake out the liquid from the wells.
b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of five washes.
d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmLy to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

Automated Wash procedure

If using an automated wash system, set the dispensing volume to 300-350/µLwell. Set the wash cycle for 5 washes with no delay between washes. If necessary, remove the microwell plate from the washer, invert over a paper towel, and tap firmLy to remove any residual wash solution from the microwells.
8. At the same rate, and in the same order as the specimens, add 100 µL of the Conjugate solution to each well, including reagent blank well.
9. Incubate the plate at room temperature (20-25°C) for 25 ±/- minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. At the same rate and in the same order as the specimens, add 100µL of TMB to each well, including reagent blank well.
12. Incubate the plate at room temperature (20-25 ℃) for 10 to 15 minutes.
13. Stop the reaction by adding 50 µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate

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several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. Read the plate within 30 minutes of the addition of the Stop Solution.

Quality Control

1. When performing the assay, test the Calibrator in triplicate. Also include a reagent blank, Negative Control, and Positive Control in each assay.
2. Calculate the mean of the three calibrator determinations. If any of the three calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two wells.
3. The mean OD value for the positive control and negative controls should fall within the following ranges:
Negative Control < 0.250
Positive Calibrator > 0.300
Positive Control > 0.500
a. The OD of the negative control divided by the mean OD of the positive calibrator should be < 0.9.
b. The OD of the positive control divided by the mean OD of the positive calibrator should be > 1.25.
c. Consider the test invalid and repeat if the results do not meet the above conditions.
4. Monitoring for substantial reagent failure using the Positive Control and Negative Control will not ensure precision at the assay cut-off.
5. If necessary, test additional controls according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Calculation

Correction Factor

The manufacturer determined a cutoff OD value for positive samples and correlated to the Calibrator. The correction factor (CF) will allow for determination of the cutoff value for positive samples. It will also correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Components List located in the kit box.

Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above. (CF x mean OD of Calibrator = cutoff OD value)

Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:

Mean OD of Calibrator = 0.793
Correction Factor = 0.25
Cut off OD = 0.793 x 0.25 = 0.198
Unknown Specimen OD = 0.432
Specimen Index Value or OD value = 0.432/0.198 = 2.18

CALCULATIONS FOR SEMI-QUANTITATIVE INTERPRETATIONS:

Conversion of Optical Density to AAU/mL*
The conversion of OD to unit value (AAU/mL) can be represented by the following equation:
Test Specimen AAU/mL = (A x B)/C where:
AAU/mL = Unknown unit value to be determined.
A = OD of the test specimen in question
B = Unit value of the positive calibrator (AAU/mL)
C = The mean OD of the calibrator

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Example:
Test specimen OD = 0.946
Calibrator OD = 0.435
Calibrator unit value = 155 AAU/mL
Test Specimen AAU/mL = (0.946 x 155) / 0.435
Test Specimen = 337 AAU/mL

Reference Values

In a study conducted by the manufacturer of 90 normal donor sera from Southwestern United States were evaluated for Myeloperoxidase autoantibodies. Of the 90 tested, none were positive. In another study using 113 specimens, which were sent to a reference laboratory in Northeastern United States, eight (8/113 = 7.1%) were positive for anti-Myeloperoxidase IgG. Taken together, these studies demonstrate that the incidence of IgG antibody to Myeloperoxidase is relatively rare.

Interpretation of Results

Index Values or OD ratios are interpreted as follows:
Index Value or OD Ration
Negative Specimens < 0.90
equivocal Specimens 0.91 to 1.09
Positive Specimens > 1.10
Use the above guidelines when evaluating or interpreting patient specimens. equivocal specimens should be repeated. Specimens which are repeatedly equivocal should be evaluated using an alternate serological method. Elevated autoantibody levels to any of the six autoantigens may be indicative of a specific rheumatic disorder. The genaeral description section of this package insert describes some of the more common diseases associated with elevated autoantibody levels.
NOTE: When interpreting the anti-Sm/RNP result to determine potential anti-RNP (only) activity, one must consider the anti-Sm and the anti-Sm/RNP result simultaneously.

Specificity

A study was performed to evaluate the assay for potential cross reactivity to other autoantibodies. Testing of eight specimens, were positive for antibodies to nuclear antigens (ANA) on HEp-2 cells, showed that two of the specimens demonstrated a homogeneous pattern, two demonstrate a nucleolar pattern, two demonstrated the centromere pattern, and two demonstrated a speckled pattern. For the summary of the results of this study, see Table 4 below. The results of this investigation indicate that cross reactivity with other antinuclear antibodies is not likely.

Reproducibility

Six specimens were tested; three positive specimens, one specimen near the cut off zone, and two negative specimens. On each of three days, a technician tested each specimen once a day, eight times each, resulting in 24 test points. A responsible party then calculated the intra-assay and inter-assay precision from the resulting data.

Precautions

1. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
2. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
3. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products

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were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institute of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens.
4. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
5. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
6. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (sodium azide, 0.1% (w/v) react with laboratory plumbing which may cause explosion on hammering.
7. The Stop Solution is TOXIC. Causes burn. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
8. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
10. Wipe bottom of plate free of residual liquid and/or fingerprints, which can alter optical density (OD) readings.
11. Dilution or adulteration of these reagents may generate erroneous results.
12. Reagents from other sources or manufacturers should not be used.
13. TMB Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test procedure, Substrate Incubation section to determine the amount of TMB to be used.
14. Never pipette by mouth. Avoid contact or reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Cross contamination of reagents and/or samples could cause erroneous results.
17. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
18. Avoid splashing or generation of aerosols.
19. Do not expose reagents to strong light during storage or incubation.
20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Wash solution should be collected in a disposal basin. Treat the waste solution with 10 household bleach (0.% sodium hypochlorite). Avoid exposure to reagents to bleach fumes.
22. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
24. Do not allow the conjugate to come in contact with containers or instruments, which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate’s enzymatic activity.
25. Do not expose any of the reactive reagents to bleach-containing solutions, or to any strong odors from bleachcontaining solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

Limitations

1. Do not make a diagnosis based on anti-MPO ELISA results alone. Interpret test results for anti-MPO in conjunction with the clinical evaluation and the results of other diagnostic procedures.

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2. The performance evaluation was conducted of this assay with lipemic, hemolyzed and icteric specimens; therefore, do not test these specimens with this assay.
3. The results of this assay are not diagnostic proof of the presence or absence of disease. Do not start Immunosuppressive therapy based on a positive result.

Analyte Gene Information

Gene Name	MPO myeloperoxidase [Homo sapiens]
Official Symbol	MPO
Synonyms	MPO; myeloperoxidase;
GeneID	4353
mRNA Refseq	NM_000250
Protein Refseq	NP_000241
MIM	606989
UniProt ID	P05164
Chromosome Location	17q21.3-q23
Pathway	C-MYB transcription factor network, organism-specific biosystem; Folate Metabolism, organism-specific biosystem; IL23-mediated signaling events, organism-specific biosystem; Phagosome, organism-specific biosystem; Phagosome, conserved biosystem; Selenium Pathway, organism-specific biosystem; Transcriptional misregulation in cancer, organism-specific biosystem;
Function	chromatin binding; heme binding; heparin binding; metal ion binding; oxidoreductase activity; peroxidase activity;

REFERENCES

1. Davies D, Moran ME, Niall JF, Ryan GB: Segmental glomerulonephritis with antineutrophil antibody: Possible arbovirus aetiology. Br. J. Med. 285:606, 1982
2. Van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA: Autoantibodies against neutrophils and monocytes: Tools for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1:425-429, 1985.
3. Feehally J, Wheeler DC, Walls J, et al: A case of microscopic polyarteritis associated with antineutrophilia cytoplasmic antibodies. Clin. Nephrol. 27: 214-215, 1987
4. Falk RJ, Becker M, Terrell R, Jennette JC: Antigen specificity of P-ANCA and C-ANCA (Abstract). The 3rd International Workshop on ANCA, ashington, DC 1990: 2-3.
5. Jennette JC, Falk RJ: Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N.Engl.J. Med. 318: 1651-1657, 1988.
6. Savage COS, Tizard J, Jayne D, et al: Anti-neutrophil cytoplasmic antibodies in Kawasaki disease. Arch. Dis. Child. 64: 360-363, 1989.
7. procedures for the collection of diagnostic blood specimens by venipuncture. Second Edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
8. procedures for the Handling and processing of Blood Specimens. NCCLS D°Cument H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
9. U.S. Department of Labor, °Ccupational Safety and Health Administration: °Ccupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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