DRG® Myeloperoxidase IgG ELISA (EIA-2776)

CE

Revised 22 Dec. 2010 rm (Vers. 2.1) IVD

INTENDED USE

The DRG 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 anti-neutrophil cytoplasmic antibodies (ANCA). MPO-ANCA may be associated with autoimmune disorders such as Wegener's granulamotosis, ICGN, MPA and PRS. This test is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Anti-neutrophil cytoplasmic antibody (ANCA) was initially described by Davies, *et al* in 1982 (1). Since this initial discovery, ANCA has been found to be associated with a number of Systemic Vasculitides (SV). ANCA is now recognized 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 (2)
Polyarteritis (3)
"Overlap" Vasculitis (4)
Idiopathic Crescentic Glomerulonephritis (ICGN) (5)
Kawasaki Disease (6)

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 ELISA ASSAY

The DRG 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 incubated in microwells coated with MPO (antigen). Anti-MPO specific IgG antibodies 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 microtiter wells 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.

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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.
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. Sample diluent. One 30 ml bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. (pH 7.2 +/- 0.2). Green solution, 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 bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
8. Stop Solution: One 15 ml bottle (red cap) containing 1M H_2S0_4 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 cap) 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.**

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. 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.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV b 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 Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (9).
5. 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.
6. 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.

7. The Diluent, Controls, Conjugate, and Wash Buffer contain sodium azide at a concentration of <0.1% (w/v). Reports of sodium azide forming lead or copper azides in laboratory plumbing showed that hammering may cause explosions. To prevent, rinse sink thoroughly with water after disposing of a solution containing sodium azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution of adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. 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 is 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.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200 µL.
- Multichannel pipette capable of accurately delivering (50-200µL).
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or plate washing system.

- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant, (Example: 10% household bleach, 0.5% sodium hypochlorite).

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C.
2. Coated microwell strips: Store between 2°C and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2°Cand 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 and Sample diluent: Store at 2-8°C.
7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store at 2 and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. 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.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (8, 9). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. Performance of testing after 8 hours requires sera to be stored between 2° and 8°C, but for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20° or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual kit components 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.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g: 10 ul of serum + 200 ul of Sample Diluent. **NOTE:** shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100 ul 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 ul of Sample Diluent to well A1 as a reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 +/- 5 minutes.
7. Wash the microwell strips 5X.

A. 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.

B. 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. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added. at the same rate and the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) 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 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. The plate should be read within 30 minutes after the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 ul/Well
3. ⟶ Incubate 20 to 30 minutes
4. Wash and add Conjhugate – 100 ul/Well
5. ⟶ Incubate 20 to 30 minutes
6. Wash and add TMB 100 ul/Well
7. ⟶ Incubate 10 to 15 minutes
8. Add Stop Solution 50 ul/Well-Mix
9. READ

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 positive calibrator determinations. If any of the three positive 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 calibrator and the OD values for the positive and negative controls should fall within the following ranges:

	OD RANGE
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. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. Calculations:

1. **Correction Factor**
   The manufacturer detrmiend 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 and to 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 data label located in the kit box.

2. **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)

3. **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

INTERPRETATIONS

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

Retest specimens with OD ration values in the equivocal range (0.91-1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

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

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

INTERPRETATION OF UNIT VALUES:
The manufacturer has established the following guidelines for interpretation of patient results:

<150 AAU/mL – Negative or Non-reactive
150 – 180 AAU/mL – Equivocal or Borderline
<180 AAU/mL – Positive or Reactive

Retest specimens with results in the equivocal range (150-180) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATION OF THE ASSAY
1. A diagnosis should not be made on the basis of anti-MPO ELISA results alone. Test results for anti-MPO should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens; therefore, these specimens should not be tested with this assay.
3. The results of this assay are not diagnostic proof of the presence or absence of disease. Immunosuppressive therapy should not be started based on a positive result.

EXPECTED RESULTS
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.

PERFORMANCE CHARACTERISTICS
COMPARATIVE STUDY
An in-house comparative study was performed to demonstrate the equivalence of the DRG International, Inc. Myeloperoxidase IgG ELISA test system to another commercially available Myeloperoxidase IgG ELISA test system.
Performance was evaluated using 316 specimens; 196 disease-state specimens, 113 specimens that were sent to a reference laboratory in the Northeastern United States for routine ANCA serology, and 7 specimens, which

were previously tested and found to be reactive for ANCA. A summary of the clinical specimens appears in Table 1 below. The results of the investigation have been summarized in Table 2 below.

Table 1: Summary of Clinical Specimens

			AGE			
n	**Male**	**Female**	**High**	**Low**	**Mean**	**Comments**
45	18	27	82	14	54.7	Disease Category: Wegener's Granulomatosis
41	21	20	100	22	63.2	Disease Category: Idiopathic Necrotizing & Crescentic Glomerulonephritis
41	16	25	87	20	63.1	Disease Category: Microscopic Polyarteritis
39	17	22	94	11	60.8	Disease Category: Pulmonary Renal Syndrome
30	15	15	78	3	43.4	Vasculitis/Glomerulonephritis Disease Controls, Non-ANCA related vasculitis.
6	Information Not Available					Previously tested ANCA positive, no diagnosis available
113	Information Not Available					Specimens sent to a reference laboratory for routine ANCA seroloogy

Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement

		DRG MPO IgG ELISA Result			
		+	-	±*	Totals
Commercial	+	93	2	1	96
MPO ELISA	-	2	195	4	201
Test System	±*	6	12	1	19
	Totals	101	209	6	316

*Equivocal specimens were excluded from all calculations.

Relative Sensitivity = 93/95 = 97.9% 95% Confidence Interval** = 95.0 to 100%

Relative Specificity = 195/197 = 98.9% 95% Confidence Interval** = 97.6 to 100%

Relative Agreement = 288/292 = 98.6% 95% Confidence Interval** = 97.3 to 99.9%

**95% confidence intervals calculated using the exact method.

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. Depiction of the results of the experiment is below.

TABLE 3	DRG international, Inc. MPO IgG ELISA: Result Precision Testing:			
Day 1: Intra-Assay Reproducibility				
Specimen	**Mean Result (AAU/mL)**	**Standard Deviation**	**Percent CV**	**Replicates**
1	2269	89	3.9	8
2	300	19	6.3	8
3	49	7	14.8	8
4	144	13	9.3	8
5	6	2	30.5	8
6	3749	116	3.1	8
Day 2: Intra-Assay Reproducibility				
Specimen	**Mean Result (AAU/mL)**	**Standard Deviation**	**Percent CV**	**Replicates**
1	2037	97	4.8	8
2	320	17	5.3	8
3	63	7	10.7	8
4	159	11	7.0	8
5	11	3	27.3	8
6	3249	114	3.5	8
Day 3: Intra-Assay Reproducibility				
Specimen	**Mean Result (AAU/mL)**	**Standard Deviation**	**Percent CV**	**Replicates**
1	2160	76	3.5	8
2	324	12	3.7	8
3	57	4	6.6	8
4	155	7	4.4	8
5	13	2	13.4	8
6	3057	67	1.9	8
Inter-Assay Reproducibility: All Days Combined				
Specimen	**Mean Result (AAU/mL)**	**Standard Deviation**	**Percent CV**	**Replicates**
1	2155	128	5.9	24
2	314	19	6.0	24
3	56	8	14.4	24
4	153	12	8.1	24
5	10	4	38.4	24
6	3501	230	6.6	24

CROSS REACTIVITY

A study was performed to evaluate the assay for potential cross reactivity to other autoantibodies. Testing of eight specimens, which 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.

Table 4. Results of the Cross Reactivity Investigation

	ANA HEp-2 IFA Results:		MPO IgG ELISA Results:	
Sample No.	**Pattern**	**Endpoint Titer**	**Optical Density**	**AAU/mL**
1	Homogeneous	1:1280	0.02	36
2	Homogeneous	1:640	0.01	15
3	Speckled	1:2560	0.02	30
4	Nucleolar	1:1280	0.01	25
5	Centromere	1:1280	0.02	28
6	Centromere	1:1280	0.01	10
7	Speckled	1:5120	0.02	30
8	Nucleolar	1:10240	0.00	5

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