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2°C-8°C



Σ=96 tests



#1681-2

ANCA SCREEN ELISA TEST SYSTEM

Catalog no.:1681-2

An Enzyme-Linked Immunosorbent Assay for the simultaneous detection of IgG

Antibodies to Myeloperoxidase (MPO) and/or Proteinase-3 (PR-3)

For *In Vitro* Diagnostic Use

INTENDED USE

The Diagnostic Automation, Inc. ANCA Screen ELISA test system is intended for the qualitative detection of anti-Myeloperoxidase and/or anti-Proteinase-3 IgG antibody 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 and/or PR3 may be associated with autoimmune disorders such as Wegener's Granulomatosis, 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 Diagnostic Automation, Inc. ANCA Screen ELISA test system is designed to detect IgG class antibodies to MPO and/or PR-3 in human sera. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in microwells coated with a mixture of MPO and PR-3 (antigen). Anti-MPO and/or anti-PR-3 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 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.

- 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.

KIT COMPONENTS

Reactive Reagents

- Twelve, 1 x 8-well strips coated with a mixture of Myeloperoxidase and Proteinase-3 enzymes (antigen). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
- Horseradish peroxidase conjugated goat anti-human IgG. Ready to use. One 30mL vial.
- Human high positive serum control. One 0.5mL vial.
- Human low positive standard. One 0.5mL vial.
- Human negative serum control. One 0.5mL vial.
- Sample diluent. One 50 mL bottle containing Tween-20, BSA, and PBS, pH 7.2 ± 0.2 . Ready to use. (Green solution with a green cap; Product Number 005N). **NOTE:** This reagent may be used with any Diagnostic Automation, Inc. ELISA test system utilizing Product Number 005N.

Reagents common to all Diagnostic Automation Inc. ELISA Test Systems

- Substrate solution. One 35 mL bottle containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use
- Stop solution. One vial containing 30mL of 1.0M H₂SO₄, 0.7M HCl. Ready to use. (Clear solution with a red cap).
- Wash Buffer. One 100mL bottle containing phosphate-buffered-saline and Tween-20. 10X concentrate. (Blue solution with a clear cap). **NOTE:** 1X solution will have a pH of 7.2 ± 0.2 .

Non-reactive Components

- Sample dilution plate. One 96 well microtiter plate for preparing serum dilutions.
- Data labels: one label is adhered to the inside lid of the kit box and a second label is inside the kit box .
- Package Insert .

PRECAUTIONS

- For *In Vitro* Diagnostic Use.
- The microwell strips do not contain any viable, infectious agents. However, the strips should be considered potentially infectious and handled accordingly. Wash solutions should be collected in a disposable basin and treated with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.
- Do not use the ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
- Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
- Control sera, conjugate, and wash buffer contain preservative which may be toxic if ingested; Thimerosal at a concentration of 0.04% (w/v). The sample diluent contains sodium azide at a concentration of 0.1% (w/v).
- Sodium azide has been reported to form lead r copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water afer disposing of solution containing sodium azide.
- Dilution or adulteration of these reagents may result in loss of sensitivity.
- Do not substitute reagents from kits with different lot numbers or from other manufacturers.
- Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, 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 Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (9).

10. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
11. Avoid microbial contamination of reagents. Incorrect results may occur.
12. Cross contamination of reagents and/or samples could cause false results.
13. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely. Do not use substrate solution if it has begun to turn blue. To help eliminate the possibility of contamination, refer to Test Procedure, Section D.1.
14. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
15. Strict adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be brought to 20-25°C before starting the assay.
16. Improper washing will cause false positive or false negative results. Be sure to blot the plates free of any residual wash solution before adding conjugate or substrate solution. Do not allow the wells to dry out between incubations.
17. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with water.
18. Caution: Liquid waste at acid pH should be neutralized before adding to sodium hypochlorite (bleach).
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. Do not allow the conjugate to come in contact with containers 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.
23. 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.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of reading at a wavelength of 450nm.
2. Microliter pipettes capable of accurately delivering 10 and 200 µL.
3. Adjustable multichannel pipette (50-200µL) for dispensing conjugate, substrate and stop solution.
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 pipette: 1, and 10 or 25 mL.
9. Disposable pipette tips.
10. Paper towels.

11. Timer with alarm capable of measuring to an accuracy of ± 1 second.
12. Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (7, 8). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° 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 which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C.
2. Antigen coated microwell strips: Extra strips should be immediately resealed with desiccant and returned to storage at 2-8°C. Strips are stable for 60 days after the envelope has been opened and properly resealed, and the indicator remains blue.
3. Peroxidase conjugated goat anti-human IgG: Store at 2-8°C. DO NOT FREEZE.
4. Human positive and negative controls: Store at 2-8°C.
5. TMB substrate solution: Store at 2-8°C.
6. Wash buffer: Store at 2-25° C. Stable for 30 days at 2-8°C after diluting to 1X, or 7 days when stored at room temperature.
7. Sample diluent: Store at 2-8°C.
8. Stop solution: Store at 2-25°C.

NOTE: All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.

PROCEDURE

PREPARATION OF REAGENTS

1. Wash Buffer: Dilute the 100mL of 10X concentrate with 900mL of distilled or deionized water. Mix thoroughly to dissolve any crystals that may be present.
2. Sample diluent, stop solution, conjugate, and substrate solutions are ready to use.

TEST PROCEDURE

A. Set-Up of the Assay

Remove the individual kit components and allow them to warm to room temperature (20-25°C). Determine the total number of samples and controls to be tested. The low positive standard

should be run in triplicate. Also, a high positive control, negative control, and a reagent blank

must be included each time the assay is run. Controls and samples should be run in duplicate until the laboratory becomes proficient with the test procedure. Each sample, control, and reagent blank requires one antigen coated microwell.

Determine the number of microwells needed. After the strips and holder have warmed to room temperature cut open the protective envelope and remove the plate containing the antigen coated microwell strips. Strips that are not needed for the assay should be placed into the re-sealable pouch, sealed and returned to storage at 2-8° C.

B. Serum Incubation

Prepare a 1:21 dilution of the positive and negative controls, the low positive standard and each patient serum as follows:

1. Add 10 μ L of each sample to a separate well of the dilution plate provided. Add 200 μ L of sample diluent to each well containing a sample.
2. Using a multichannel pipette, transfer 100 μ L of each diluted sample and control from the dilution plate to the test plate. Withdraw and expel the samples several times before the final transfer to ensure that the samples are properly mixed. Use a different pipette tip for each sample. Add 100 μ L of sample diluent to a well as a reagent blank.
3. Cover the wells with a plate sealer and incubate the plate at room temperature (20-25°C) for 20 to 22 minutes.
4. Wash the microwell strips 5X.
 - 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 (10% household bleach) at the end of the days run.

NOTE: Autowash - If using an automated wash system, set the dispensing volume to 300-350 μ L/well. Set the wash cycle for 5 washes with no delay between washes. Remove microtiter plate from washer, invert plate over paper towel and tap firmly to remove any residual wash solution from the wells.

C. Conjugate Incubation

1. Add 100 μ L of the conjugate solution to each well at the same rate and in the same order as the specimens were added.
2. Incubate the plate at room temperature (20-25°C) for 20 to 22 minutes.
3. Wash the plate by following the procedure in Step **B.4., a.** through **d.**

D. Substrate Incubation

1. Add 100 μ L of the TMB substrate solution to each well at the same rate and in the same order as the conjugate was added. (One mL of TMB substrate is sufficient for 8 wells).
2. Incubate the plate at room temperature (20-25°C) for 10 to 12 minutes.
3. Add 50 μ L of stop solution to each well at the same rate and the same order as the TMB solution was added. Positive samples will turn from blue to yellow. After adding stop solution, **tap plate several times to ensure that the samples are thoroughly mixed.**
4. Set the microplate 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.

QUALITY CONTROL

1. Each time the assay is run, the low positive standard (LPS) should be run in triplicate. A high positive and negative control, and reagent blank must also be included in each assay.
2. Calculate the mean of the three low positive determinations. If any of the three low positive values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the LPS and the OD values for the high positive and negative controls should fall within the following ranges:

OD RANGE

Negative Control ≤ 0.25

Low Positive Standard ≥ 0.30

High Positive Control ≥ 0.50

- a. The OD of the negative control divided by the mean OD of the LPS should be ≤ 0.9 .
 - b. The OD of the high positive control divided by the mean LPS value should be ≥ 1.25 .
 - c. If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated.
4. The HPC is intended to monitor for substantial reagent failure and 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.

INTERPRETATION OF RESULTS**A. Calculations****1. Correction Factor**

A cutoff OD value for positive samples has been determined by Diagnostic Automation, Inc. and correlated to the low positive standard (LPS). The correction factor (CF) will allow you to determine 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 LPS vial.

2. Cutoff OD Value

To obtain the cutoff OD value, multiply the correction factor by the mean LPS value determined above.

3. OD Ratios

Calculate an OD ratio for each specimen by dividing its OD value by the cutoff OD from Step 2. OD ratios are interpreted as follows:

OD RATIO

Non-Reactive Specimens ≤ 0.90

Reactive Specimens ≥ 1.10

Equivocal Specimens 0.91 - 1.09

B. Interpretation

1. An OD ratio ≤ 0.90 indicates no detectable IgG antibodies to Myeloperoxidase or Proteinase-3.
2. An OD ratio ≥ 1.10 is reactive for IgG antibodies to Myeloperoxidase and/or Proteinase-3. The results of this test system are qualitative; ratio values in the reactive range are not indicative of the amount of antibody present
3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be re-tested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made on the basis of ANCA Screen ELISA results alone. Test results for ANCA Screen 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. Although the ANCA Screen will detect antibodies to both MPO and PR-3, the assay will not differentiate between the two. Positive ANCA Screen specimens should be tested on the Diagnostic Automation, Inc. individual MPO and PR-3 ELISAs to determine which antibody is present.
4. 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 Diagnostic Automation, 90 normal donor sera from Northeastern United States were evaluated for ANCA autoantibodies. Of the 90 tested, one (1.1%) was positive and one (1.1%) was equivocal.

In another study using 105 specimens which were sent to a reference laboratory in Northeastern United States, fourteen (14/105 = 13.3%) were positive for ANCA antibodies. Taken together, these studies demonstrate that the incidence of ANCA is relatively rare.

PERFORMANCE CHARACTERISTICS

Comparative Study:

An in-house comparative study was performed to demonstrate the equivalence of the Diagnostic Automation, Inc. ANCA Screen IgG ELISA test system to another commercially available ANCA IgG ELISA test system. Performance was evaluated using 316 specimens; 196 disease-state specimens, 113 specimens which 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. The results of the investigation have been summarized in Tables 1 and 2 below:

Table 1. Summary of Clinical Specimens

n			AGE			Comments
	Male	Female	High	Low	Mean	
45	18	27	82	14	54.7	Disease Category: Wegener's Granulomatosis
41	21	20	100	22	63.2	Disease Category: Idiopathic Necrotizing and 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.
7	Information Not Available					Previously tested ANCA positive, no diagnosis available
113	Information Not Available					Specimens sent to a reference laboratory for routine ANCA serology

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

Diagnostic Automation, Inc. ANCA Screen IgG ELISA Result					
		+	-	±*	Totals
Commercial	+	148	8	0	156
ANCA ELISA	-	3	113	4	120
Test System	±*	15	22	3	40
	Totals	166	143	7	316

*Equivocal specimens were excluded from all calculations.

Relative Sensitivity = $148/156 = 94.9\%$ 95% Confidence Interval** = 91.4 to 98.3%

Relative Specificity = $113/116 = 97.4\%$ 95% Confidence Interval** = 94.5 to 100%

Relative Agreement = $261/272 = 96.0\%$ 95% Confidence Interval** = 93.6 to 98.3%

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

Reproducibility:

To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested, eight replicates each, on each of three days. These results were then used to calculate mean unit values, standard deviations, and percent CV. Two of the specimens were strong positives, two were clearly negative, and two were near the assay cut off. The results of the study have been summarized below.

Table 3.**Diagnostic Automation, Inc. ANCA Screen: Results of Precision Testing:**

Day 1: Intra-Assay Reproducibility.				
Specimen	Mean Ratio	Standard Deviation	Percent CV	Replicates
1	7.40	0.25	3.4	8
2	5.89	0.20	3.4	8
3	1.00	0.07	6.7	8
4	0.97	0.07	7.6	8
5	0.17	0.01	5.3	8
6	0.08	0.01	6.0	8

Day 2: Intra-Assay Reproducibility.				
Specimen	Mean Ratio	Standard Deviation	Percent CV	Replicates
1	7.10	0.21	3.0	8
2	5.59	0.30	5.4	8
3	0.90	0.05	5.6	8
4	0.78	0.06	7.7	8
5	0.18	0.02	8.3	8
6	0.06	0.01	20.7	8

Day 3: Intra-Assay Reproducibility.				
Specimen	Mean Ratio	Standard Deviation	Percent CV	Replicates
1	7.60	0.30	3.9	8
2	5.90	0.32	5.4	8
3	1.14	0.07	6.1	8
4	0.96	0.07	7.3	8
5	0.21	0.03	11.9	8
6	0.08	0.01	12.5	8

Inter-Assay Reproducibility: All Days Combined.				
Specimen	Mean Ratio	Standard Deviation	Percent CV	Replicates
1	7.40	0.34	4.6	24
2	5.80	0.30	5.2	24
3	1.00	0.11	11.0	24
4	0.90	0.11	12.2	24
5	0.18	0.02	11.1	24
6	0.07	0.02	21.4	24

Cross Reactivity:

To evaluate the test system for potential cross-reactivity to other autoantibodies, eight specimens which were positive for antibodies to nuclear antigens (ANA) on HEp-2 cells were tested. Two of the specimens demonstrated a homogenous pattern, two demonstrated a nucleolar pattern, two demonstrated the centromere pattern, and two demonstrated a speckled pattern. The results of this study have been summarized in 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.

Sample Number	ANA HEp-2 IFA Results		ANCA Screen ELISA Results:	
	Pattern	Endpoint Titer	Optical Density	Ratio
1	Homogenous	1:1280	0.066	0.36
2	Homogenous	1:640	0.019	0.10
3	Speckled	1:2560	0.044	0.24
4	Nucleolar	1:1280	0.101	0.56
5	Centromere	1:1280	0.050	0.28
6	Centromere	1:1280	0.035	0.19
7	Speckled	1:5120	0.051	0.28
8	Nucleolar	1:10240	0.028	0.15

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