

INTENDED USE

The MESACUP TEST MPO is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-myeloperoxidase (MPO) antibodies in human serum. The MESACUP TEST MPO is intended for in vitro diagnostic use as an aid in the diagnosis of certain systemic vasculitides such as microscopic polyarteritis and crescentic glomerulonephritis.

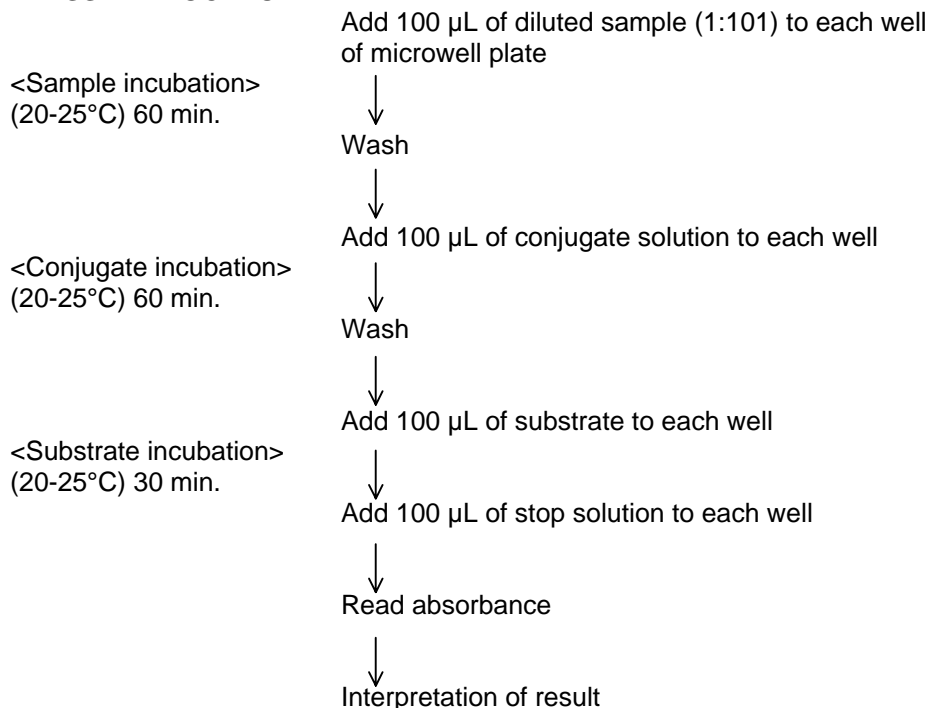
SUMMARY AND EXPLANATION

In 1982 Davis et al. described the presence of antineutrophil cytoplasmic antibodies (ANCA) in serum of patients with necrotising glomerulonephritis. The use of indirect immunofluorescence (IIF) identified two distinct pattern types described in 1988 by Falk et al, pANCA (perinuclear) and cANCA (cytoplasmic), in renal patients with systemic vasculitis. The major pANCA target antigen is myeloperoxidase and the major cANCA target antigen is proteinase III (PR-3). Several other antigens (lactoferrin, elastase, cathepsin G) are associated with ANCA reactivity to lesser degree. Although the use of an ELISA can identify the presence of antibodies specific for MPO and PR-3, it is still recommended to use an IIF method for patient screening. Testing positive IIF samples by ELISA can provide useful additional information. ELISA assays that identify the presence of anti-MPO and anti-PR-3 antibodies can be used not only to confirm IIF positive patterns, but also distinguish specific systemic vasculitides. Anti-PR-3 antibodies are detected in the majority of active Wegner's granulomatosis patients and only occasionally positive for anti-MPO. Diseases such as microscopic polyarteritis and crescentic glomerulonephritis are more likely associated with the presence of anti-MPO antibodies.

PRINCIPLE

The MESACUP TEST MPO measures IgG anti-MPO antibodies present in human serum by ELISA. Calibrators and patient serum are added to microwells coated with MPO antigens, allowing anti-MPO antibodies to react with the immobilized antigen (Sample incubation). After washing to remove any unbound serum proteins, horseradish peroxidase conjugated anti-human IgG is added and incubated (Conjugate incubation). Following another washing step, the peroxidase substrate is added and incubates for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The assay can be quantified by measuring the reaction photometrically and calculating the results.

BRIEF ASSAY PROCEDURE



REAGENTS AND STORAGE

MPO MICROWELL STRIPS 96 wells (twelve 1 x 8 well strips) coated with MPO antigens produced from native purified proteins. The breakaway strips are packaged in a frame and sealed in a foil envelope with desiccant and are stable at 2-8°C until the labeled expiration date.

CALIBRATOR 1 (0 U/mL)* Two vials containing 1.5 mL of Assay Diluent. Ready to use; make no further dilution. Stable at 2-8°C until labeled expiration date.

CALIBRATOR 2 (100 U/mL) Two vials containing 1.5 mL of anti-MPO antibody positive human serum in Assay Diluent. Ready to use; make no further dilution. Stable at 2-8°C until labeled expiration date.

CONJUGATE REAGENT One vial containing 15 mL of horseradish peroxidase conjugated goat anti-human IgG, HEPES, Proclin 150, and BSA. Ready to use. Stable at 2-8°C until labeled expiration date.

ASSAY DILUENT* Two 50 mL bottles containing PBS, Tween 20, and 0.1% sodium azide. Ready to use. Stable at 2-8°C until labeled expiration date.

WASH CONCENTRATE (10X)* One 100 mL bottle containing PBS and Tween-20 as a 10X concentrate. Stable at 2-8°C until labeled expiration date.

SUBSTRATE SOLUTION* One 20 mL bottle containing 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/ H₂O₂). Ready to use. Stable at 2-8°C until labeled expiration date.

STOP SOLUTION* One 20 mL bottle containing 1N sulfuric acid. Ready to use. Stable at 2-8°C until labeled expiration date.

POSITIVE CONTROL SERUM One vial containing 0.2 mL of anti-MPO antibody positive human serum with 0.1% sodium azide. Stable at 2-8°C until labeled expiration date.

NEGATIVE CONTROL SERUM One vial containing 0.2 mL of anti-MPO antibody negative human serum with 0.1% sodium azide. Stable at 2-8°C until labeled expiration date.

*These reagents can also be used for MESACUP-2 TEST Sm, MESACUP-2 TEST RNP, MESACUP-2 TEST SS-A, MESACUP-2 TEST SS-B, MESACUP-2 TEST Scl-70, MESACUP-2 TEST JO-1, MESACUP-2 TEST CENP-B and MESACUP TEST PR-3.

PRECAUTIONS

1. This product is for in vitro diagnostic use only.
2. Do not use kit components beyond the stated expiration dates.
3. Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed with plenty of water. TMB contains irritant and Stop Solution consists of a 1N sulfuric acid.
4. Calibrators are derived from human serum in which HBs antigen, HCV antibody, and HIV antibody have not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis, or any other infectious diseases.
5. Calibrator 1, Calibrator 2, Controls and Assay Diluent contain sodium azide (0.1%) as a preservative. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush with plenty of water when disposing of materials containing sodium azide into a drain.
6. Matching lot numbers of microwell strips, conjugate and calibrator 2 must be used together in the assay. Do not substitute reagents from other kits.
7. All reagents must be brought to room temperature (20 -25°C) before starting the assay.
8. Do not expose the kit to direct sun light during assay and storage.
9. Avoid microbial and cross contamination of reagents or samples.
10. Incubation temperatures above or below normal room temperature (20-25°C), shorter or longer incubation periods, and inaccurate dilution may give erroneous results.
11. The wells must be rinsed with Wash Solution properly to avoid a false positive.
12. Carefully pipette each sample and reagent to avoid cross contamination between microwells.
13. All microwell strips which are not immediately required should be returned to the zipper pouch, which must be carefully resealed to avoid moisture absorption.
14. Wash concentrate may become turbid at 2-8°C, which does not cause inconsistent results.
15. Implements used for the test should be discarded or treated as described here. Soak in 2% final conc. glutaraldehyde solution for at least 1 hour or soak in 0.5% sodium hypochlorite solution (available chlorine: approx. 5000ppm) for at least 1 hour or autoclave at 121°C for a minimum of 20 minutes.

SPECIMEN COLLECTION AND PREPARATION

Serum is the preferred sample matrix. Blood should be collected by venipuncture, and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, specimens should be stored at 2 to 8°C. If specimens are to be stored for more than 72 hours, they should be frozen at -20°C or below. Avoid repeated freezing and thawing. Avoid using hemolyzed, icteric, or lipemic serum as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader [wavelength: 450 nm (primary), 620 nm (reference)]
- Multichannel micropipette (e.g., 100 µL - 300 µL) for dispensing conjugate, substrate, and stop solution.
- Single channel pipettes (10 µL, 100 µL and 1 mL)
- Reagent reservoirs
- Automated plate washer or wash bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of wash solution
- Test tubes for patient sample dilutions (e.g. 1000 µL)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

PROCEDURE

PREPARATION OF REAGENTS

Bring all assay materials to room temperature (20-25°C) prior to use. Microwell Strips: Remove sufficient microwell strips from pouch and place them in the frame. Promptly return unused strips to refrigerated storage. Wash Solution: Prepare 1:10 dilution of the Wash Concentrate prior to use (i.e., add 100 mL of Wash Concentrate to 900 mL of distilled water). The diluted wash solution is stable for 2 weeks at 2 -8°C. Do not dilute Calibrator 1, Calibrator 2, Conjugate Reagent, Assay Diluent, Substrate or Stop Solution, which are ready-to-use.

PREPARATION OF SAMPLES

Dilute each patient serum and control sample 1:101 by adding 10µL of serum to 1mL of Assay Diluent. Diluted samples must be used within a day. Diluted samples can also be used for MESACUP TEST PR-3, MESACUP-2 Test RNP, MESACUP-2 Test Sm, MESACUP-2 Test SS-A, MESACUP-2 Test SS-B, MESACUP-2 Test Scl-70, MESACUP-2 Test Jo-1, MESACUP-2 Test CENP-B, MESACUP-2 Test Mitochondria M2.

ASSAY PROCEDURE

1) SAMPLE INCUBATION Using a multi-channel pipette, transfer 100µL of each diluted sample, Positive and Negative Controls into the appropriate microwells of the antigen test plate. Add Calibrators directly to appropriate wells. Do not dilute Calibrators. Incubation starts upon pipetting to the antigen-coated microwells. Pipetting should be completed as quickly as possible. Cover wells with a plate sealer and incubate for 60 minutes at room temperature (20-25°C).

2) WASHING Aspirate or discard the well contents. Fill all wells with Wash Solution and then completely aspirate or discard the contents. Wash 4 times. After the last wash, tap the plate on a paper towel to remove any remaining wash solution. When an automated plate washer is used, wash 4 times. Each laboratory is recommended to confirm its own appropriate washing times and setup. Wash Solution should be used at 20-25°C.

3) CONJUGATE INCUBATION Pour Conjugate Reagent into a reservoir. Add 100 µL of the Conjugate Reagent to each well with a multichannel pipette. Cover wells with the plate sealer and incubate for 60 minutes at room temperature (20-25°C).

4) WASHING Wash the microplate following the STEP 2 procedure.

5) SUBSTRATE INCUBATION Pour Substrate into a reservoir and pipette 100 µL of the Substrate to each well with a multichannel pipette. The reservoir should be different from the one, which was used for the Conjugate Reagent. A new disposable reservoir should be used because Substrate is easily oxidized by metal ion. The Substrate, once poured in a reservoir, should not be returned to the bottle. Cover wells with the plate sealer and incubate for 30 minutes at room temperature (20-25°C).

6) STOP REACTION Pour Stop Solution into a reservoir. Pipette 100 µL of the solution to each well with a multichannel pipette.

7) READING Read the absorbance of each well at 450 nm. If a dual wavelength plate reader is available, set the test wavelength at 450 nm and the reference wavelength at 620 nm. Reading should be done as quickly as possible after stopping the reaction. Ensure that the bottom of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading the plate.

CALCULATION OF RESULTS

$$\text{Unit value (U/mL)} = \frac{(A_{450}\langle\text{Sample}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)}{(A_{450}\langle\text{Calibrator 2}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)} \times 100$$

*A₄₅₀ is abbreviation of absorbance value at 450 nm.

EXAMPLE:

A₄₅₀ Calibrator 1 = 0.050
A₄₅₀ Calibrator 2 = 1.000
A₄₅₀ Sample = 1.200

$$\text{Sample value} = \frac{1.200 - 0.050}{1.000 - 0.050} \times 100 = 121 \text{ U/mL}$$

QUALITY CONTROL

- 1) Controls and calibrators should be included with each assay to ensure that all kit reagents and procedures perform properly.
- 2) Additional controls may be included with each assay according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
- 3) The user should refer to NCCLS Document C24-A for additional guidance on appropriate quality control practices.
- 4) The mean absorbance of Calibrator 1 should be ≤ 0.100 and Calibrator 2 should be ≥ 0.500. Failure to achieve these values may indicate that the kit is no longer suitable for use.
- 5) The mean value for the negative control should be < 22 U/mL and positive control should be > 50 U/mL.

If any of these criteria are not met, the results are invalid and the test should be repeated. Before repeating assay, check the following procedure steps:

- Incubation Temperature
- Incubation Time
- Washing
- Sample dilutions

TEST INTERPRETATION AND EXPECTED VALUE

The following is intended only as a guide for interpretation. Each laboratory is recommended to establish its own criteria for test interpretation based on sample populations typically encountered.

Anti-MPO Value (U/mL)	Interpretation
< 22	Negative for anti-MPO Ab
≥ 22	Positive for anti-MPO Ab

An international reference material for anti-MPO was not available while this assay was developed. The assay is calibrated in relative arbitrary units (U/mL). The assay cutoff value was established by testing a population with systemic vasculitis patients. Results were compared with immunofluorescence assay results to determine at what value the best overall accuracy was achieved (combination of highest relative specificity and sensitivity). A positive result indicates the presence of anti-MPO antibodies and suggests the possibility of systemic vasculitis. A negative result indicates lack of anti-MPO antibodies or levels below the negative cutoff of the assay. It is recommended that laboratories report results with a similar statement: "Results were determined using the RhiGene MESACUP Test MPO assay. Results from other manufacturers' assay methods should not be used interchangeably. The ELISA values cannot be correlated with endpoint titers of other methods."

LIMITATIONS

As with other diagnostic test procedures, the results obtained with the MESACUP Test MPO serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedures. Not all systemic vasculitis patients are positive for MPO antibodies, may have antibodies to other antigens, or may not have detectable antibodies to MPO at all. The use of specimen types other than serum have not been characterized and are not recommended. Specimens that contain immune complexes may cause non-specific binding.

NORMAL RANGE

A population of eighty (80) healthy donor specimens was tested on the MESACUP Test MPO. Using a cutoff value of 22 U/mL, all 80 specimens resulted negative on the MESACUP assay for a clinical specificity of 100%.

RELATIVE SENSITIVITY AND SPECIFICITY

To determine the relative sensitivity and specificity of the assay, 159 samples were tested by the MESACUP Test MPO and another commercial ELISA method for ANCA. Of the 159 samples, 126 were negative and 26 were positive by both methods. Three samples were positive by the MESACUP Test but not the other ELISA method. These same three samples tested positive by IFA for a p-ANCA pattern. Four samples were negative by the MESACUP Test and weakly positive by the reference method.

		MESACUP Test MPO			
		Neg	Pos		
Reference Method	Neg	126	3	Relative Specificity	98%
	Pos	4	26	Relative Sensitivity	87%
				Relative Agreement	96%

CLINICAL SENSITIVITY AND SPECIFICITY

Serum samples from a population of seventy nine (79) patients suspected to have a systemic vasculitis disease were tested on the MESACUP Test MPO and by an IIF ANCA method. Positive IIF specimens were identified for pANCA or cANCA pattern. Sixty five (65) samples were positive by IIF, and thirty seven (37) of these were determined to have a pANCA pattern. Of those, twenty seven (27) were positive for anti-MPO by the MESACUP assay. Six of the ten pANCA positive specimens that were negative on the MESACUP Test MPO were also negative by another ELISA kit for anti-MPO and the other four specimens were weak positive (10.3, 10.8, 11.3, 12.8 U/mL; cut off value = 9 U/mL).

In an additional study, serum samples from various autoimmune disease groups were tested to further determine clinical specificity of the MESACUP Test MPO assay. These groups included Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's Syndrome (SjS), Polymyositis / Dermatomyositis (PM/DM), and Rheumatoid Arthritis (RA). The table below summarizes the results from both of these studies and a healthy donor population.

	Healthy	SLE	MCTD	SjS	PM/DM	RA	Vasculitis pANCA -	Vasculitis pANCA +
# of Samples (n)	80	10	10	10	10	9	42	37
Mean value (U/mL)	1.1	7.5	5.4	9.6	3.2	0.3	6.8	91.9
Positive Rate (%)	0%	10%	0%	0%	0%	0%	5%	73%

PRECISION AND REPRODUCIBILITY

Three lots of the MESACUP Test MPO were tested to determine the intra-assay, inter-assay and inter-lot value precision. Variability was determined by testing each of two samples (low positive and high positive), on three separate assay runs with three different plate lots. Results are presented in the table below.

Anti-MPO Specimens	Mean Intra-assay CV	Mean Inter-assay CV	Inter-lot
Weak positive (26.3 U/mL)	3.8 %	7.0%	5.5 %
High positive (130.3 U/mL)	3.4 %	6.3%	3.5 %

ASSAY RANGE

The assay range of this kit is from 5 U/mL to 200 U/mL.

INTERFERING SUBSTANCES

Hemoglobin (up to 440 mg/dL), bilirubin C (up to 19.5 mg/dL), bilirubin F (up to 18.6 mg/dL), chyle (up to 2,350 units as Formazine) and/or Rheumatoid Factor (up to 500 IU/mL) have no affect on the assay results, but it is recommended to avoid using highly hemolysed or lipemic samples.

REFERENCES

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