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ISO 13485-2003



IVD



See external label



2°C-8°C



Σ=96 Tests

REF

1420-1

Mitochondria IgG,M,A ELISA

Cat. No. 1420-1

INTENDED USE

The Diagnostic Automation, Inc. (DAI) Mitochondria Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitation of antibodies to mitochondria in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of primary biliary cirrhosis. For *in vitro* diagnostic use.

INTRODUCTION

Systemic autoimmune disease is characterized by the presence of circulating auto-antibodies directed to a wide variety of cellular antigens (1,2,3). Systemic lupus erythematosus (SLE), commonly referred to as Lupus is the best known of these diseases. Other possible connective tissue diseases include mixed connective tissue disease (MCTD), Sjogren syndrome, scleroderma, and polymyositis/dermatomyositis. The majority can be diagnosed by clinical presentation and their antibody profiles to the various antigens involved, which include dsDNA, SM, RNP, Ro, La, Scl-70, Jo-1 and Histones. Therefore, immunoassays for autoantibodies are useful for diagnostic and prognostic evaluations of autoimmune disease (1,2,3).

Mitochondria (M2) antigen is part of the pyruvate dehydrogenase complex (4). Approximately 90-95% of patients with primary biliary cirrhosis (PBC) have antibodies to M2. Antimitochondria antibodies occur occasionally in other liver conditions and scleroderma. The antibody is rarely seen in other conditions (5,6).

Classically, antibodies to autoimmune antigens are detected by double immunodiffusion. However, the test is lengthy and suffers weak sensitivity. Enzyme-Linked Immunosorbent Assays (ELISAs) combine greater sensitivity with ease of use. Many ELISAs have been developed and validated for detecting autoantibodies to various antigens (7,8,9,10).

PRINCIPLE OF THE TEST

The DAI Mitochondria test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgM, and IgA antibodies to mitochondria antigens. Purified Mitochondria antigens are attached to a solid phase microassay well. Diluted test sera are added to each well. If the antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG, M, A, is added to each well. If antibody is present the conjugate will bind to the antigen-antibody complexes. After incubation the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen.

KIT COMPONENTS

1. **Mitochondria antigen coated microassay plate:** 96 well, provided with a strip holder and stored in a foil bag with desiccant and humidity indicator card.
2. **Serum Diluent:** One bottle, 30 mL. Contains buffer, BSA and Tween 20, and proclin (0.1%) as preservative..
3. **High Positive Control:** One vial, 0.4 mL. Contains human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.1%) 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.
4. **Calibrator:** One vial, 0.4 mL. Contains human serum with antibodies that react with the antigen used to calibrate the assay. Kit specific Correction Factor printed on vial label.
5. **Negative Control:** One vial, 0.4 mL. Contains human sera or defibrinated. Sodium azide (0.1%) and pen/strep (0.1%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay.
6. **Low Positive Control:** One vial, 0.4 mL. Contains human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.1%) added as preservatives, with established range printed on vial label. The Low Positive Control is utilized to control the range near the cutoff of the assay.
7. **Wash Buffer (20x concentrate) :**One bottle, 50 mL. Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween 80 and proclin (0.1%) as a preservative..
8. **Horse Radish Peroxidase (HRP) Conjugate:** One bottle, 15 mL. Goat anti-human IgG, IgM and IgA containing prolin (0.1%) and gentamicin as preservatives. Ready to use.
9. **Chromogen/Substrate Solution:** One bottle, 15 mL. Contains 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use. Reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
10. **Stop Solution:** One bottle, 15 mL. Contains a H₂SO₄ solution. Ready to use.

REAGENT STORAGE CONDITIONS

1. All kit components that are stored at their recommended storage conditions are stable until the expiration date on their label. Do not use past their expiration date.
2. Antigen coated wells. Unused strips should be immediately resealed in the foil bags with desiccant and humidity indicator card and returned to storage at 2-8° C. If the bag is resealed with tape the wells are stable for 30 days. If the bag is resealed with a heat sealer the wells are stable until their expiration.
3. All other reagents are stored at 2-8° C in their original containers.
4. 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.

PRECAUTIONS

1. Each donor unit used in the preparation of the Calibrator and Controls was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative. Because no test method can offer complete assurance that human immunodeficiency virus (HIV-1), hepatitis B virus, or other infectious agents are absent, these specimen/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 Institute of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1993 and FDA labeling guidelines for *in vitro* diagnostic reagent manufactures, sec. 1985. (11).
2. Certain reagents in this kit contain sodium azide for use as a preservative. Azides may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water to minimize azide build up.
3. This product is for IN VITRO DIAGNOSTIC USE only.
4. Reagents contain preservatives which may be toxic if ingested.
5. Do not pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
6. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with copious quantities of water.
7. Avoid splashing or generation of aerosols.
8. Do not use heat inactivated sera.
9. Do not mix or interchange reagents between lots of kits or from other manufacturer.
10. Do not dilute or adulterate kit reagents.
11. Do not cross contaminate reagents or specimens.
12. Do not use TMB Substrate solution if it has begun to turn blue.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Do not vary reagent and incubation temperatures above or below room temperature (21 - 25° C).
15. Washing is important. Improperly washed wells will give erroneous results. Do not allow the well to dry out between incubations.

SPECIMEN COLLECTION

1. Aseptically collect blood samples by venipuncture and prepare serum using accepted technique (12).
2. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
3. Sera may be stored up to five days at 2-8° C. If a further delay in testing is needed store frozen at -20 to -70° C in a non-defrosting freezer. Avoid multiple freeze/thaw of patient samples.
4. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
5. Do not heat inactivate sera.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water, CAP Type 1 or equivalent.
9. Timer capable of measuring to an accuracy of +/- 1 second.
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL H₂O).
11. 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 operators' manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

PREPARATION OF REAGENTS

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

TEST PROCEDURE

1. Determine the number of patients to be assayed. For each assay the Calibrator should be run in duplicate. Also the High Positive Control, Low Positive Control, Negative Control, and a reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations.

Example Configuration:

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

2. For each test serum, Calibrator and Control to be assayed prepare a 1:21 serum dilution. Add 10 μ L of each serum sample to 200 μ L of Serum Diluent. Mix well.
 3. To individual wells, add 100 μ L of the appropriate diluted Calibrator, Controls and patient sera. Add 100 μ L of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent requirements for the correct reagent blank well configuration.
 4. Incubate each well at room temperature (21-25) $^{\circ}$ C for **15 minutes +/- 1 minutes**.
 5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 μ L of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling 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 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.
- ** 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 semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 μ L) is recommended. A total of up to five (5) washes may be necessary with automated 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.**
6. Add 100 μ L Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
 7. Incubate each well at room temperature (21-25) $^{\circ}$ C for **30 minutes +/- 1 minutes**.
 8. Repeat wash as described in Step 5.
 9. Add 100 μ L Chromogen/Substrate Solution (TMB) to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
 10. Incubate each well at room temperature (21-25) $^{\circ}$ C for **15 minutes +/- 2 minutes**.
 11. Stop reaction by addition of 100 μ L of Stop Solution (1N Sulfuric Acid) following the same order of Chromogen/Substrate (TMB) addition, including the reagent blank well. Tap the plate gently along the outside, to mix contents of the wells. Wait a **minimum of 5 minutes** and read. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
 12. 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.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrators and Controls must be run with each test run.
2. Reagent Blank must be < 0.150 O.D. at 450 nm (when read against Air Blank)
3. The mean O.D. value for the Calibrator should be ≥ 0.300 at 450nm (when read against Reagent Blank).
4. The index values for the High Positive, Low Positive, 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 should be repeated.
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 C24A for guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
7. If above criteria are not met on repeat, contact Diagnostic Automation Inc Technical Services.

INTERPRETATION

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

Example : O.D.s obtained for Calibrator = 0.38, 0.42
 Mean O.D. for Calibrator = 0.40
 O.D. obtained for patient sera = 0.60
 Correction factor = 0.50
 Cutoff value = 0.50 x 0.40 = 0.20
 Index Value = 0.60/0.20 = 3.00

ANALYSIS

1. The patients' Index Values are interpreted as follows:

<u>Index Value</u>	<u>Results</u>	<u>Interpretation</u>
≤ 0.90	Negative	No detectable antibody to Mitochondria by the Elisa test
0.91 - 1.09	Equivocal	Samples should be retested. See number 2 below.
≥ 1.10	Positive	Indicates presence of detectable antibody to Mitochondria by the Elisa test.

2. Specimens with index values in the equivocal range should be retested. If still equivocal retest by an alternate method or test a new sample.

EXPECTED VALUES

1. From 1-5% of apparently normal individuals may contain autoantibodies (1,2,3).
2. Antibodies to M2 are found in approximately 90-95% of patients with primary biliary cirrhosis (PBC) (5,6).
3. Antimitochondria antibodies occur occasionally in other liver conditions and scleroderma (5,6).
4. Antibodies to mitochondria are rarely seen in other conditions (5,6).

LIMITATIONS

1. The result of the assay should not be interpreted as being diagnostic. The results should only be used as an aid to diagnosis. The results should be interpreted in conjunction with the clinical evaluation of the patient.
2. Sera from patients with other autoimmune diseases and from normal individuals may contain autoantibodies.
3. The assay should be used only with serum. Icteric, lipemic, hemolyzed and heat inactivated serum should be avoided.
4. Index Values of ≥ 10.00 should be reported as greater than 10.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

The DAI Mitochondria ELISA kit was evaluated relative to a commercially available ELISA test kit. Table 1 summarizes the data.

Table 1
Sensitivity and Specificity of the DAI Mitochondria ELISA Kit

		<u>DAI Mitochondria ELISA Kit</u>			
		<u>Positive</u> <u>≥ 1.10</u>	<u>Equivocal</u> <u>0.91-1.09</u>	<u>Negative</u> <u>≤ 0.90</u>	<u>Total</u>
Alternate	Positive >25	17	0	0	17
Elisa	Equivocal .21-.25	0	0	0	0
Kit	Negative < 21	0	2	122	124
	Total	17	2	122	141

Sera falling in the equivocal range were excluded from the following calculations

Relative Sensitivity = 17/17 = 100%
Relative Specificity = 122/122 = 100 %
Relative Agreement = 139/139 = 100 %

PRECISION

The precision of the DAI Mitochondria ELISA kit was determined by testing seven different sera eight times each on three different assays. The data is summarized in Table 2. With proper technique the user should obtain C.V.'s of less than 15%.

Table 2
Precision Data

Serum #	X	Assay 1 (n=8)		X	Assay 2 (n=8)		X	Assay 3 (n=8)		X	Inter Assay (n=24)	
		S.D.	C.V.		S.D.	C.V.		S.D.	C.V.		S.D.	C.V.
1	17.70	.587	3.32%	17.00	.103	6.07%	15.84	.899	5.68%	16.85	1.14	6.77%
2	16.09	.532	3.31%	15.69	.804	5.12%	15.00	.396	2.64%	15.59	.735	4.71%
3	16.59	.703	4.24%	16.67	.590	3.36%	15.76	1.46	9.26%	16.34	1.04	6.36%
4	2.36	.285	12.1%	2.28	.269	11.8%	2.43	.251	10.3%	2.36	.264	11.2%
5	4.71	.597	12.7%	4.41	.518	11.8%	5.23	.535	10.2%	4.78	.630	13.2%
6	.290	.092	31.7%	.251	.169	67.3%	.264	.115	43.6%	.268	.124	46.3%
7	.330	.141	42.7%	.373	.179	48.0%	.404	.205	50.7%	.369	.172	46.6%

X = Mean Mitochondria Value S.D. = Standard Deviation C.V. = Coefficient of Variation

LINEARITY

The Mitochondria index values were determined for serial twofold dilutions of five positive sera. The index values were compared to \log_2 of dilution by standard linear regression. The data in Table 3 indicates that the assay is semi-quantitative.

Table 3
Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	r^2
1	5.71	5.18	4.79	3.93	3.36	2.21	1.29	.790	.984
2	7.79	6.07	3.57	2.07	1.36	0.64		.948	
3	3.00	2.29	1.86	1.50	1.36	1.14	0.86		.938
4	3.36	2.64	2.07	1.57	1.21	0.93		.974	
5	4.09	3.78	2.89	2.27	2.09	1.86	1.43	1.13	.957

r^2 = coefficient of determination. Linear regression compared Mitochondria Index Value to \log_2 of dilution.

CROSS REACTIVITY

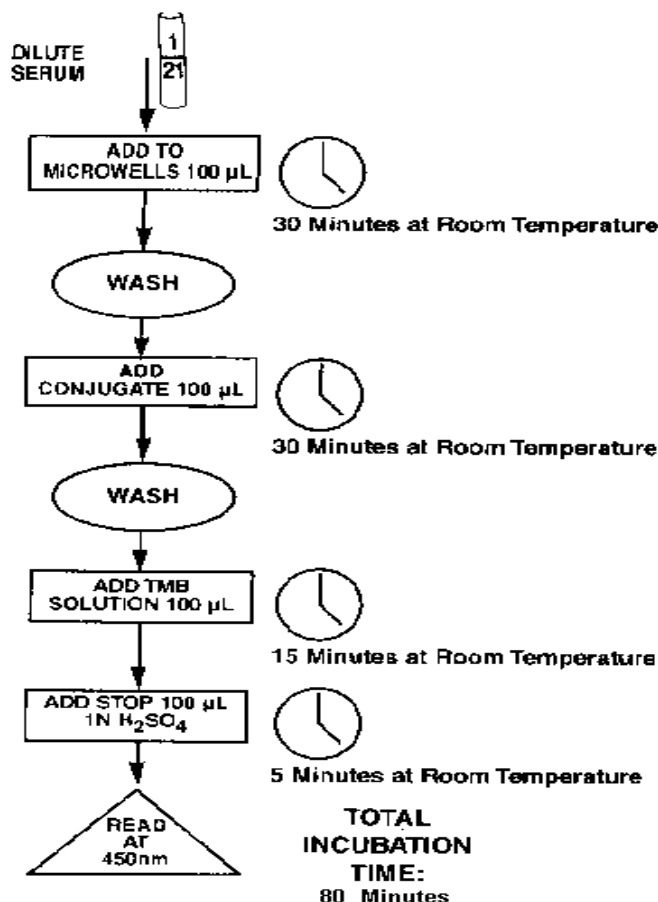
Sera containing high level of antibodies to potentially cross reactive antigens were assayed on the DAI Mitochondria ELISA kit. The data in Table 4 indicate that antibodies to alternate autoimmune antigens do not cross react with the DAI Mitochondria ELISA kit.


Table 4
Cross Reactive Data

Serum #	Antibody Specificity	Mitochondria Index Value	Interpretation
1	SM	0.39	-
2	SM	0.04	-
3	SM	0.06	-
4	RNP	0.25	-
5	RNP	0.07	-
6	RNP	0.01	-
7	Ro	0.18	-
8	Ro	0.82	-
9	Ro	0.02	-
10	La	0.23	-
11	La	0.36	-
12	La	0.07	-
13	Scl-70	0.47	-
14	Scl-70	0.13	-
15	Scl-70	0.18	-
16	Jo-1	0.40	-
17	Jo-1	0.36	-
18	Jo-1	0.10	-
19	DsDNA	0.16	-
20	DsDNA	0.34	-
21	DsDNA	0.11	-

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