IMTEC-AMA-M2

AMA M2
ELISA for the Quantitative Determination of Anti-Mitochondrial Antibodies M2 (IgG)

Package Size

REF	ITC60040	96 Tests	Complete Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL DB13, WASH 20x WB03, SUB TMB ELISA and STOP STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.

All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits.

Store reagents at 2...8°C.

Intended Use

IMTEC-AMA M2 is an indirect solid-phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against mitochondrial antigens in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of primary biliary cirrhosis.

Primary biliary cirrhosis (PBC) is a chronic inflammatory liver disease. It starts with inflammatory changes in the small and in the medium sized biliary ducts and slowly develops pathological changes in the tissue, leading to complete liver cirrhosis.

Antimitochondrial antibodies (AMA) directed against the inner and outer mitochondrial membranes are highly specific for PBC.

Antibodies directed to the underlying antigen M2 (AMA M2) can be detected in about 90% of all patients with PBC.

Principle

The test is based on the immobilisation of the pyruvate dehydrogenase complex (PDH) to the solid phase of microtiter strips and subsequent binding of AMA M2 from patient serum. The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

Reagents and Contents

MTP	12	**Microtiter Strips** (in 1 strip holder) 8-well snap-off strips, ready for use coated with PDH	
CAL	1 – 5 5 x 1.5 ml	**Calibrators IgG** (white cap), human serum, inked according to concentration, ready for use AMA M2 level: 2 U/ml **(1)**, 8 U/ml **(2)**, 31 U/ml **(3)**, 125 U/ml **(4)**, 500 U/ml **(5)**	
NC	1.5 ml	**Negative Control Serum** (green cap), human, ready for use	
PC	1.5 ml	**Positive Control Serum** (red cap), human, ready for use Concentrations are stated on the labels.	
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) Concentrate (20x) for 1 l TRIS buffer	pH 6.9 ± 0.2
DIL DB13	100 ml	**Dilution Buffer** (blue cap) ready for use Phosphate buffer	pH 6.8 ± 0.2
CON	15 ml	**Conjugate Solution** (white cap) anti-human-IgG HRP conjugate, ready for use	
SUB TMB ELISA	15 ml	**TMB solution** (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin Hydrogen peroxide	pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l

++++ Change of 📖 ++++ Please read marked text carefully! ++++

STOP STOP ELISA	15 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	1	**Adhesive Strip**	

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

Reagent Preparation

Allow the testkit and all its components to reach room temperature before use! Used bottles should be closed carefully and stored at 2...8°C. Store SUB protected from light.

Do not use polystyrene vessels for handling of CON.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

Specimen

Patient sera

Use samples freshly collected or freeze samples at –20°C. **Freeze and thaw once only.** Do not use serum samples inactivated by heat treatment at 56°C.

Allow the samples to reach room temperature (30 min.).

Dilute sera 1:101 with DIL (add 10 µl serum to 1 ml DIL).

Procedure

- **Pipette 100 µl** diluted sample, CAL, PC and NC into MTP, for blank use DIL instead of sample dilution, seal MTP with adhesive strip.
- Incubate for **1 hour** at RT.
- Discard the solution from MTP. Wash MTP 3 times using 300 µl WASH per well.
- Discard WASH and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl** CON and seal MTP with adhesive strip.
- Incubate for **30 min**. at RT.
- Discard the solution from MTP. Wash MTP 3 times using 300 µl WASH per well.
- Discard WASH and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl** SUB and incubate for **10 min.**. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 min..
- **Add 100 µl** STOP per well.
- Read absorbance values at 450 nm within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.

Automation

The IMTEC-AMA M2 ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated prior to diagnostic use.

Validation of the test

The test results are valid provided the following criteria are met for the obtained results:

- PC is within the indicated range (see label).
- NC is lower than the cut-off-value of the test.
- CAL 5 does not fall below an absorbance value of 0.6.
- The absorbances of CAL 1-5 keep raising.

In order to improve accuracy of the test results we recommend to run CAL 1-5, PC, NC and patient samples in duplicate.

Interpretation of Results

Plot measured absorbances against units of CAL 1-5 in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of antimitochondrial antibodies in the patient samples can be determined.

Results between 5-10 U/ml are equivocal and above 10 U/ml (cut-off value) positive.

Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated antimitochondrial antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

The performance characteristics for this assay have not been established for plasma samples.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/el-60040.pdf or

www.human-de.com/data/gb/vr/el-60040.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

STOP **Warning**

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

SUB **Danger**

· Hazard statements

H360D May damage the unborn child.

· Precautionary statements

CAL NC PC WASH 20x DIL CON SUB STOP

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

Klafki M. *et al.*, Posterabstract C11. 3, Dresden Symposium on Autoantibodies **71**, Dresden 1996

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