IMTEC-ANA-LIA Maxx

Line Immuno Assay (LIA) for the Detection of Antinuclear Antibodies (dsDNA, Nucleosome, Histone, SmD1, PCNA, P0, SS-A/Ro 60, SS-A/Ro 52, SS-B/La, CENP-B, Scl70, U1-snRNP, AMA M2, Jo-1, PM-Scl, Mi-2 and Ku)

Package Size

REF	ITC92005	24 Tests	Complete Testkit

IVD

Please read the instructions carefully before testing

Intended Use

IMTEC-ANA-LIA MAXX is an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against dsDNA, Nucleosome, Histones, SmD1, PCNA, ribosomal P0 (RPP), SS-A/Ro 60, SS-A/Ro 52, SS-B/La, CENP-B, Scl70, U1-snRNP, AMA M2, Jo-1, PM-Scl, Mi-2 and Ku in human serum or plasma. The assay is intended for in vitro diagnostic use only.

The IMTEC-ANA-LIA MAXX provides a differential diagnosis using 17 different autoantibodies. The specific bands are arranged on each test strip according to their relevance in dedicated diseases (SLE, Sjögren-Syndrome, CREST-syndrome, Sclerodermie, MCTD, PBC and Myositis). This provides an additional diagnostic survey of autoimmune diseases and identification of overlap syndromes.

Antinuclear antibodies (ANA) are autoantibodies of different specificity directed against antigens of the cell nucleus. The detection of ANA and ENA (extractable nuclear antigens) antibodies is important for diagnosis of collagenosis especially of systemic lupus erythematosus (SLE) and the "mixed connective tissue disease (MCTD)" that is strongly associated with SLE, and rheumatic diseases.

Anti mitochondrial antibodies (AMA) to M2-antigen are specific for PBC and can be detected in 90% of PBC patients. Anti-AMA M2 antibodies also appear frequently in collagenosis prior to clinical symptoms.

Jo1, PM-Scl, Mi-2, and Ku are diagnostic markers of poly- and dermatomyositis as well as myositis associated autoimmune diseases and overlap-syndromes.

Principle

The test is based on the principle of the line immuno assay (LIA). Nuclear and associated cytosolic antigens are applied as lines on a nitrocellulose membrane:

antigens	identity
dsDNA	native
Nucleosome	native
Histone	native
SmD1	peptide
PCNA	recombinant
P0 (RPP)	recombinant
SS-A/Ro 60	native
SS-A/Ro 52	recombinant
SS-B/La	recombinant
CENP-B	recombinant
Scl70	recombinant
U1-snRNP	recombinant
AMA-M2	native
Jo-1	recombinant
PM-Scl	recombinant
Mi-2	recombinant
Ku	recombinant

The nitrocellulose membrane is blocked to prevent unspecific reactions. During incubation of a strip with diluted patient samples autoantibodies present in the sample will bind to the antigens on the strip. For the detection of the bound antibodies a secondary horseradish peroxidase (HRP)-labelled anti-human IgG antibody is used. After addition of the substrate and stop solution the appearance of brown lines indicate the existence of (auto) antibodies against the respective antigen.

Kit Content

STRIP	24	**Test Strips** (orange colour coding) coated with antigen (see table), ready for use
DIL LIA	3 Bottles	**Powder** for the preparation of 30 ml dilution buffer (blue cap)
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) concentrate (20x) for 1 l buffer
CON	29 ml	**Conjugate Solution** (white cap) anti-human-IgG conjugate, ready for use
SUB LIA	30 ml	**Substrate Solution** (black cap), ready for use colourless to bluish 3,3', 5,5'-tetramethylbenzidine 1.2 mmol/l hydrogen peroxide 2.4 mmol/l
STOP LIA	26 ml	**Stop Solution** (red cap) sulphuric acid, ready for use 0.1 mol/l
	2 pcs.	**Incubation Tray**
	1 pc. each	**Scoring sheet, Tweezers, bonding sheet,** transparent **Evaluation Template**

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens should be handled as potentially infectious. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

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

STOP LIA, SUB LIA can irritate eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

When stored at 2...8°C unopened vials are stable until the expiry date.

After reconstitution, DIL LIA and WASH and opened CON are stable for 6 weeks at 2...8°C.

Store SUB LIA protected from light.

Precautions ⚠

DIL LIA, WASH 20x WB03 and SUB LIA may be interchanged between lots and LIA 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.

Do not use polystyrene vessels for handling of CON.

Any crystallised salt of WASH 20x inside the bottle must be resolved before use.

Do not dry STRIP during the incubation steps.

Do not touch STRIP with fingers, use tweezers.

Remove diluted samples completely after incubation of STRIP to avoid cross contamination.

Specimen, Controls

Serum and plasma with the anticoagulants citrate or EDTA.

Do not use highly lipemic, hemolysed or icteric specimens.

Undiluted specimens may be stored for 5 days at 2...8°C, or for one year at -20°C**. Freeze and thaw once only.** Thawed specimen should be carefully homogenised. Eliminate particulate matter by centrifugation or filtration.

Reagent preparation

Bring all reagents to **room temperature** (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Washing Buffer Solution WASH

Dilute 1 part WASH 20x with 19 parts distilled water.

Dilution buffer Solution DIL LIA

Dissolve the content of one bottle DIL LIA with 30 ml of WASH and agitate well.

Procedure

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high band intensity.

W1: Remove liquids completely.

W2: Add WASH and incubate for 5 min with gentle agitation.

W3: After washing, remove remaining liquid.

Pipetting Scheme

Follow the procedure exactly as described. Pay particular attention to the washing procedure!	
⚠ Reagents and specimens should be at room temperature before use. **⚠ Use rocking shaker during all incubation steps.**	
Sample Preparation: Dilute specimen 1:101 with reconstituted DIL LIA (10 µl serum + 1 ml DIL LIA) 1 ml is needed for each well.	
Step 1	***Well [ml]***
Insert STRIP into the incubation tray colour coding facing up	--
WASH to wet the membrane	1
Incubate 1 min. at room temperature	
Remove WASH	
Step 2	
Diluted samples	1
Incubate 30 min. at room temperature	
Wash 3 times as described (see W1 - W3)	
WASH	1
Step 3	
CON	1
Incubate 30 min. at room temperature	
Wash 3 times as described (see W1 - W3)	
WASH	1
Step 4	
SUB LIA	1
Incubate 10 min. at room temperature	
Remove SUB LIA	
Step 5	
Add distilled water	1
Incubate 1 min. at room temperature	
Remove destilled water	
STOP LIA	1
Incubate 5 min. at room temperature	
Remove STOP LIA	
Dry STRIP thoroughly	

Automation

The IMTEC-ANA-LIA MAXX may be processed with suitable automated Blot analyzers. Applications have to be validated in prior to diagnostic use. For automated interpretation of LIA strips use HumaScan (REF ITC02851).

Test Validation

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

- Function control is visible.
- Cut-off control is visible.
- Intensity function control > intensity cut-off control

Interpretation of Results

Fix STRIP onto scoring sheet and align the reference line of the STRIP with the reference line on the scoring sheet.

Align the dotted reference line of the evaluation template with the reference line of the STRIP.

The interpretation of the test results takes place exclusively on basis of the respective cut-off control regarded for each STRIP:

The test result is **negative**, if no band is to be recognised or if the band exhibits a smaller intensity in comparison to the cut-off control.

The test is **equivocal**, if the intensity of the band and the intensity of the cut-off control do not significantly differ.

The test result is **positive**, if a band exhibits a stronger staining in comparison to the cut-off control.

Record the respective test results on the scoring sheet.

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.

The intensity of the band colour does not necessarily correlate with antibody titres as obtained with other reference methodologies. Samples from apparent normal blood donors may contain autoantibodies.

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.

Performance Characteristics

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

www.human.de/data/gb/vr/la-92005.pdf or

www.human-de.com/data/gb/vr/la-92005.pdf

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

Note

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

STOP **Warning**

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

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

Conrad K. *et al.*, Autoantibodies in Systemic Autoimmune Diseases – A Diagnostic Reference; Pabst Science Publishers, Lengerich, Berlin, Riga, Rom, Viernheim, Wien, Zagreb, 2002

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