IMTEC-Myositis-LIA

Myositis-LIA
Line Immuno Assay (LIA) for the Detection of Antibodies in Autoimmune Myositis (IgG) (Jo1, Mi2, PM-Scl, U1-snRNP, Ku)

Package Size

REF ITC60200 24 Tests Complete Testkit

IVD

Please read the instructions carefully before testing.

Intended Use

IMTEC-Myositis-LIA is an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against Jo1, Mi2, PM-Scl, U1-snRNP and Ku in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of poly- and dermatomyositis and myositis-associated overlap syndromes.

Anti-Jo1 antibodies can be detected in 33% of patients with primary polymyositis and in 25% of cases with primary dermatomyositis. Antibodies directed against Jo1 are considered as marker antibodies of a subset of myositis with lung disease.

Anti-Mi2 antibodies are detectable in 10-15% of patients with acute dermatomyositis, associated with a diagnostic specificity of >96% for the disease.

Anti-PM-Scl antibodies are found almost exclusively in patients with idiopathic myositis and/or myositis overlap syndrome or scleroderma. When found, they occur only solely. The rate of recognition by PM-Scl antibodies is 100% for the PM-Scl-100 protein and 50 to 60% for the PM-Scl-75 protein

Anti-U1-snRNP antibodies are considered to be a diagnostic marker of mixed connective tissue disease (MCTD), which is also referred to as "Sharp's syndrome". Used in this indication, the antibodies achieve a sensitivity of 100% (per definition) and a specificity of 98% in the absence of both anti-Sm and anti-dsDNA antibodies.

Anti-Ku antibodies are detectable in approx. 5-25% of patients with polymyositis/scleroderma overlap syndrome and lower frequencies in patients with primary pulmonary hypertension, SLE (in combination with other ANA specificities), primary Sjögren's syndrome and infrequently in other connective tissue diseases. Overall Ku antibodies are detectable in 1-7% of patients with myositis.

Principle

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

antigens	identity
Jo1	recombinant
Mi2	recombinant
PM-Scl	recombinant
U1-snRNP	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.

++++ - Read carefully! ++++ New Assay design++++

Kit Content

STRIP	24	**Test Strips** (blue 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'-tetramethylbenzidin 1.2 mmol/l hydrogen peroxide 2.4 mmol/l
STOPLIA	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 or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

STOPLIA, SUBLIA 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.

Use rocking shaker during all incubation steps.

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 at 2...8°C for up to 5 days, 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.	
Sample Preparation: Dilute specimen 1:101 with reconstituted 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-MYOSITIS-LIA may be processed with suitable automated Blot analyzers. Applications have to be validated in prior to diagnostic use.

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-60200.pdf or

www.human-de.com/data/gb/vr/la-60200.pdf

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.)

Colour coding

The colour coding attached above the reference serves the identification of the available IMTEC-LIA-tests:

colour coding	IMTEC-LIA test
yellow	ANA-LIA
orange	ANA-LIA-Maxx
blue	Myositis-LIA
brown	Liver-LIA S
purple	Vasculitis-LIA
black	Gastro-LIA

References

Conrad K. *et al.*, Autoantibodies in Systemic Autoimmune Diseases – A Diagnostic Reference; Pabst Science Publishers, Lengerich, 2008

LA-60200 INF ITC60200 GB 04-2014-13M

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