HOB-LIA-VASCULITIS

Line Immuno Assay (LIA) for the Detection of Antibodies in Autoimmune Vasculitis

For Qualitative Analysis of Antibodies in Autoimmune Vasculitis in Human Serum

REF MB00044; Vasculitis-3, 16 Tests
REF MB00041; Vasculitis-2, 16 Tests

INTENDED USE
The **HOB-ANCA-LIA** is for the qualitative measurement of IgG class antibodies against PR3, MPO and GBM in human serum. The assay is intended for *in vitro* diagnostic use only as an aid in the diagnosis of systemic vasculitis, i.e. Wegener's granulomatosis, and the Goodpasture syndrome.

Proteinase 3 (PR3) is the main target of cytoplasmic anti-neutrophil cytoplasm antibodies (cANCA). In contrast to that, perinuclear ANCA (pANCA) mainly react with myeloperoxidase (MPO).

cANCA are closely related to Wegener's granulomatosis classically causing severe glomerulonephritis. Repeated examination for cANCA is therefore of value for monitoring of disease activity and effect of treatment.

pANCA, detected by indirect immunofluorescence, can also be found in a lot of diseases apart from vasculitis. Consequently the detection of cANCA and pANCA by indirect immune fluorescence is not sufficient to proof systemic necrotising vasculitis. Therefore it is necessary to analyze the fine specification of PR3-ANCA and MPO-ANCA by ELISA as a second step or in parallel.

Anti-GBM antibodies (anti-glomerular basement membrane antibodies) can be detected in about 90% of patients with Goodpasture's syndrome. While Goodpasture's syndrome is a relatively rare condition (0.5% of all patients with renal diseases), it is rapidly progressive and, if not treated, fatal in 75-90%. An early diagnosis and an immediate and correct treatment decrease the lethality dramatically.

ASSAY PRINCIPLE
The test is based on the principle of the line immune assay (LIA).

PR3, MPO and GBM autoantigens are applied as lines on a nitrocellulose membrane. 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, an alkaline phosphatase labeled anti-human IgG antibody is used. After addition of the substrate solution, the appearance of purple blue lines indicates the existence of (auto) antibodies against the respective antigens.

MATERIALS PROVIDED
1, ***Test Strips*** coated with autoantigens, ready for use
……..1 x 16strips for 16T; STRIP

2, ***Sample Diluent*** consisting of TBS buffer (pH7.2±0.2), ready for use
…….....1 x 100mL for 16T; DIL

3, ***Wash Buffer Concentrate*** consisting of a 10x concentrate of TBS buffer (pH7.5±0.2), dilute with distilled or de-ionized (D.I.) water before us. BUF WASH 10x
……..1 x 50mL for 16T;
4, ***Conjugate Solution*** consisting of a 10x concentrate of anti-human-IgG ALP conjugate, diluted with sample diluents before use CONJ 10X
……..1 x 3mL for 16T;
5, ***Substrate Solution*** consisting of NBT/BCIP, ready for use
……1 x 30mL for 16T; SUBS
6, ***Scoring Sheet***
……1pcsfor 16T;
7, ***Incubation Tray***
……2pcs for 16T;
8, ***Instruction for Use***
……1pcs for16T;

LOT Lot Number


Store at 2-8℃
IVD For *In-Vitro* Diagnostic Use
Expiration Date

DETAILS OF AUTOANTIGENS COATED ON STRIP FOR DIFFERENT TYPES.
MPO, PR3 & GBM

SHELF LIFE AND STORAGE
Kit is stored at 2-8°C until stated expiration date. Do not freeze any kit component. Bring all the test kit reagents to room temperature (18~25°C) before use. Be careful to avoid the reagents to be polluted which will cause incorrect test results.

The shelf life is 18 months under proper storage conditions. Do not use any kits beyond the stated expiration date.

Kit reagents unsealed should be sealed after use and stored at 2-8°C. Unsealed kit reagents are stable for 2 months.

SPECIMEN COLLECTION
Use fresh patient specimens only or freeze samples at -20°C. Freeze samples only one time prior to use. Do not use 56°C heat inactivated samples.

SAMPLE PREPARATION

Dilute serum samples 1:101 with Sample Diluent. **For example, dilute 20µL of sample in 2mL of Sample Diluent.**

REAGENT PREPARATION AND STORAGE
Attention!

Allow the test kit and all its components to reach room temperature before use.

Used bottles should be closed carefully and stored at 2-8°C.

Unused strips should be sealed into the aluminum pouches together with desiccant.

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

Wash Buffer Concentrate
Any crystallized salt inside the bottle must be resolved before use. Dilute 1 part with 9 parts distilled or purified water. Diluted wash buffer is stable for 6 weeks stored at 2-8°C. RCNS H2O

Conjugate Solution
Pipette certain quantity of Anti-human-IgG ALP Conjugate needed and dilute 1:10 with Sample Diluent. If a test strip needs to be incubated, add 1.35mL of Sample Diluent into 0.15mL of Anti-human-IgG ALP Conjugate. Diluted Anti-human-IgG ALP Conjugate should be used out within 1 day.

RCNS	DIL

ASSAY PROCEDURE
Attention!
Do not let test strip dry during the incubation steps.

Do not touch test strip with fingers, use tweezers.

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

Please shake test strips at room temperature with gentle agitation during all incubation steps.

All details are valid per strip or patient sample.

1. Put test trip into the incubation tray, the side with color coding faces up. Add 1.5mL of Sample Diluent into each incubation tray, and incubate the test strip for 5 minutes at room temperature with gentle agitation, and then remove it.
2. Pipette 1.5mL of diluted patient sample and add it into the incubation tray, incubate for 30 minutes at room temperature with gentle agitation.
3. Remove diluted samples completely. Wash test strip 3 times using 1.5mL Wash Buffer for 5 minutes with gentle agitation. Remove Wash Buffer after every washing step.
4. Pipette 1.5mL diluted conjugate and incubate for 30 minutes at room temperature with gentle agitation.
5. Repeat step 3.
6. Remove conjugate. Add 1.5mL Substrate and incubate for 10 minutes at room temperature with gentle agitation.
7. Remove Substrate. Wash with 1.5mL distilled or purified water for 1 minute at room temperature with gentle agitation. Repeat this wash step for another 2 times.
8. Dry test strip, and stick it onto the Scoring Sheet to save the test results.

VALIDATION OF THE TEST

The test results are valid provided the following criteria are met for each strip.

1, A normal test run is indicated by a visible function control.

2, The cut-off control must be visible too.

3, Intensity function control ＞intensity cut-off control.

REFERENCE RANGE
The line immune assay (LIA) is a qualitative test method and no reference range is provided. Proportion for diluting patient samples is 1:101.

INTERPRETATION OF RESULTS
Score the test results according to the coloring intensity of the strip as negative, equivocal and positive.

The test result is negative, if no band is to be recognized 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.
Note: Patient samples which are hemolysis with hemoglobin concentration as 5mg/mL, blood-fat with triglyceride concentrations as 20mg/mL and jaundice with bilirubin concentration as 0.4mg/mL have no influence to the test results.

ILLUSTRATION OF THE STRIP

LIMITATIONS
1. The intensity of the band color indicating positive results does not necessarily correlate with antibody titers.
2. A positive result must be used in associated with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

PERFORMANCE CHARACTERISTICS
1. Coincidence rate with negative reference is proved by test as 100%.
2. Coincidence rate with positive reference is proved by test as 100%.
3. Limit of detection should not be higher than the values as below:
   PR3：0.8U/mL
   MPO：6U/mL
   GBM：5U/mL

4. Reproducibility
Test the reference reagent for 10 times. Both the test results and coloring are same, which are consistent to corresponding autoantigen type.

5. Inter-lot variation
Test one sample with kits of 3 different lots. Both the test results and coloring are same, which are consistent to corresponding autoantigen type.

6. Stability
The shelf life is 18 months under storage conditions of 2-8°C.Test the kit that is beyond the stated expiry date, the coincidence rate with negative and positive reference, limit of detection and reproducibility should confirm to each formulated performance indicator respectively.

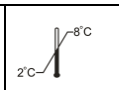
WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. Read the instruction for use carefully before use.
2. Do not use components exceeding the expiry date.
3. Do not combine reagents of other suppliers or kit components of different lots with this kit.
4. 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.
5. All components of this kit should be handled as potentially infectious. Test strips are coated with recombinant and purified native non-anthropogenic extracts from animal tissues, and alkaline phosphatase conjugated goat anti-human IgG.

REFERENCES

1. Conrad K. et al., Autoantibodies in Systemic Autoimmune Diseases-A Diagnostic Reference; Pabst Science Publisher, Lengerich, Berlin, Riga, Rom, viernheim, Wien, Zagreb, (2002)
2. Hellmark T. et al., Kidney Int. 46, 823-829, (1994)

SYMBOLS

						
Lot-number	European conformity	Authorized Representa-tive in the European	Sufficient For <n> tests	For *In-Vitro* Diagnostic use	Temperature Limit	Use before
						
Catalogue Number	Consult instructions for use	Refer accompany-ing documents	Do not use when Package is damaged	Do not Re-use	Manufactured by	

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