CE IVD 2℃ 8℃

Please read instructions for use carefully before starting the assay

AI-LFA *MPO*

Lateral Flow Assay for the qualitative determination of Myeloperoxidase specific Autoantibodies in human serum or plasma in combination with AI-LFA Reader

Single cassette REF 186026 Σ 10

BACKGROUND

Vasculitis occurs due to inflammation of blood vessel walls and exhibits many different clinical pictures, of which antineutrophil cytoplasmic antibodies (ANCA) associated small vessel vasculitis is one of the most common causes. ANCA associated vasculitis includes microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome and drug induced vasculitis. The sensitive and specific detection of Proteinease 3 (Pr3) and Myeloperoxidase (MPO) specific autoantibodies is highly recommended even at the slightest suspicion of renal vasculitis occurring in about 80% of all Wegners granulomatosis cases. Only fast and adequate treatment can avoid the development of renal failure. MPO is stored in azurophilic granules of the neutrophil granulocytes. It has an important role in the microbicidal activity of phagocytes. MPO produces hypochlorous acid from hydrogen peroxide and chloride anions during the neutrophil's respiratory burst.
The new Autoimmune Lateral Flow Assay (AI-LFA) takes use of liquid phase antigens in a most native form, thereby offering highest sensitivity and specificity

INTENDED USE

AI-LFA MPO (**A**utoimmune **L**ateral **F**low **A**ssay) is a rapid assay for the qualitative determination of MPO specific Immunoglobulin G (sIgG) in human serum or plasma. AI-LFA enables the user to perform a specific autoimmune test very fast and reliable.

PRINCIPLE

AI LFA *MPO* is available as single-strip cassette (REF 186026 10 pcs ≙ 10 det.) – plus antigen solution.
To perform the test the patient´s sample is transferred to the sample application point of the *Basis Set*. Immediately afterwards, the desired antigen solution is applied. During incubation of 20-25 min the liquid is driven through the device by capillary flow. The antigen specific IgG of the sample binds specifically to its corresponding antigen in the solution. The labelled antigens are retained at the test line (T) by a capture molecule. At the same time, the sIgG bound to the antigen is bound by an antibody coupled to coloured particles (conjugate). The intensity of the colour reaction at the test line is proportional to the amount of immune complexes consisting of ligand tagged antigen, sIgG, and IgG specific conjugate. **The signal intensity ranges from faintly pink (low titre of sIgG) to dark ruby (high titre of sIgG).**
Access conjugate, which is not bound at the test line, forms a dark ruby control line (C) after 20-25 min of incubation.

KIT COMPONENTS

AI-LFA MPO:	Basis Set (ready to use): Single-strip cassette (REF 186026;10 pcs ≙ 10 det.)
Storage:	At 2-8℃, do not freeze! Use immediately after opening the foil pouch!
Shelf life:	To expiry date if stored in foil pouch (unopened).
MPO-LFA *Antigen*:	Ready to use solution.
Storage:	At 2-8℃, do not freeze!
Shelf life:	To expiry date.

MATERIAL TO BE ORDERED SEPARATELY

AI-LFA *Reader* : REF **190002**

MATERIAL NEEDED BUT NOT PROVIDED

- micro pipette and pipette tips for 10 µL
- capillary or transfer pipette for 10 µL
- tubes for serum preparation if required
- equipment for blood withdrawal
- clock

SPECIMEN COLLECTION & PREPARATION

Either serum or plasma can be used with this assay. The use of haemolytic or lipemic specimens must be avoided.

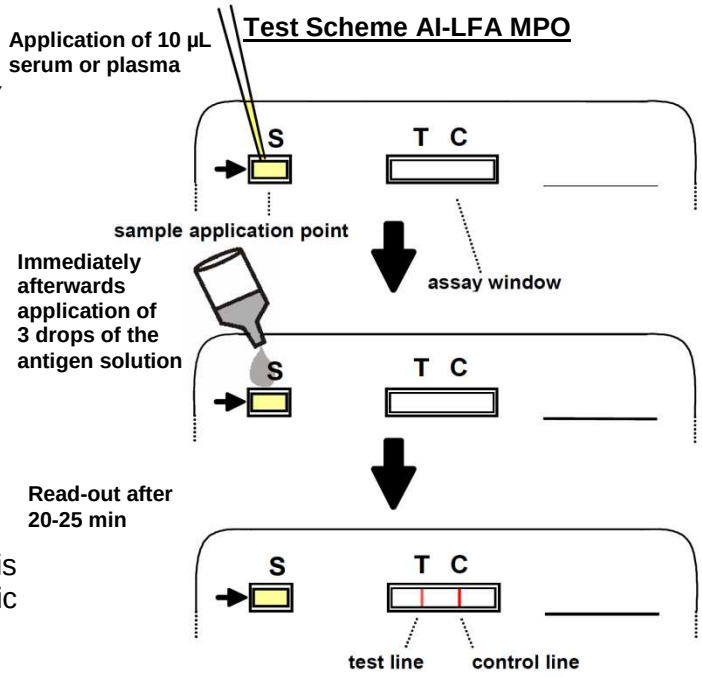
If serum or plasma is used, proceed as follows: No additives or preservatives are necessary to maintain the integrity of the specimens if stored at 2-8℃ and assayed within 48 hours after collection. If this is not possible or if specimens have to be shipped, samples must be frozen. To perform the assay, thaw and bring samples up to room temperature (RT, 18-25℃). Repeated freezing and thawing should be avoided.

ASSAY PROCEDURE

Attention!

Bring *Basis Set* (in foil pouch) as well as the antigen solution up to RT, at least 1 hour before starting the assay.

- If the foil pouch is damaged, do not use the test unit.
- Use the test unit within 30 min after opening the foil pouch.
- Avoid performing the test in direct sunlight.
- Keep Basis Set in a horizontal position and avoid movement of the cassette while running the assay.



1. Transfer **10 µL of serum or plasma** to the sample application point of the *Basis Set*.
2. Immediately afterwards, apply **3 drops** of the **antigen solution** to the sample application point of the *Basis Set* (hold dropper bottle 1 cm above the sample application point).
3. The test result becomes visible in the evaluation window after 20-25 min of incubation.

The test result has to be interpreted within 20-25 min after application of the antigen solution. Any assessment done later or earlier can cause erroneous interpretation of the result!

Attention! Only interpret Test results with AI-LFA Reader! The test result is only valid, if the control line (C) of the test unit is clearly visible!The results of AI-LFA have to be evaluated on the basis of the signal intensity of the test line (T). The intensity of the colour correlates with the amount of sIgG in the sample.

Short Operating instructions AI-LFA *READER (*opTrilyzer® plus*)*

⇨ Measurement of single-strip cassette

- to switch on AI-LFA Reader, press the button on the back of the device for 1 second
- wait until main menu appears
- √= enter, ↑↓ = navigation buttons for up and down, Esc = back
- select **Eject** and press √ to continue. Adapter for test cartridge (slot) removes automatically.
- select **Test** and press √ to continue

⇨ Single-strip cassette

- select **AI-LFA 1x** and press √ to continue
- **Antigen and Patient Selection** appears

press √ to select Antigen and patient ID

- **create / select patient** appears
- ***select*** a patient ID of the list using the # key and press √ to continue.
- ***or***
- ***create*** new patient ID with the keyboard and press √ to continue. Switch input mode between numeric and alpha-numeric with ✱. (note: to create patient data files please follow the instructions described under point **Patient data**)
- **select Antigen** appears
- select an Antigen with ↑↓ or scroll with → and ←
- press √ to continue
- **Antigen assignment finished** appears
- press √ to continue or [ESC] to edit selection
- **create / select operator** appears
- select (using #) or create an operator press √ to continue
- press √ to continue (yellow display appears)
- insert the single cassette in the slot (please note: the direction of the cassette is pictured at the slot)
- insert the slot (plus test cassette) into the Reader
- press √ to start the measurement
- test result appears
- press √ to save the result, # to print the result, or Esc to go back

⇨ Patient data (create or recall patient data files)

- - Select **Patient data** and press √ to continue
- - **Edit patient data** appears
- → select **create new patient data** and press √ to continue (for navigation follow
- the instructions, shown on display)
- Press √ to add the *patient-ID*

Press twice √ to add the *last name*
Press twice √ to add the *first name*
Press twice √ to add the *day of birth*
Press twice √ to add the *month of birth*
Press twice √ to add the *year of birth*
Press √
save patient data with √

- → select an existing patient ID and press √ to show **patient measurement results**
- → select an existing patient ID and press # to edit patient's data

⇨ Setting

- → Select **Settings** and press √ to continue
- select language
- add date and time
- delete patient data

⇨ Switch off

- To switch off AI-LFA Reader, press the button on the back of the device for 2 seconds

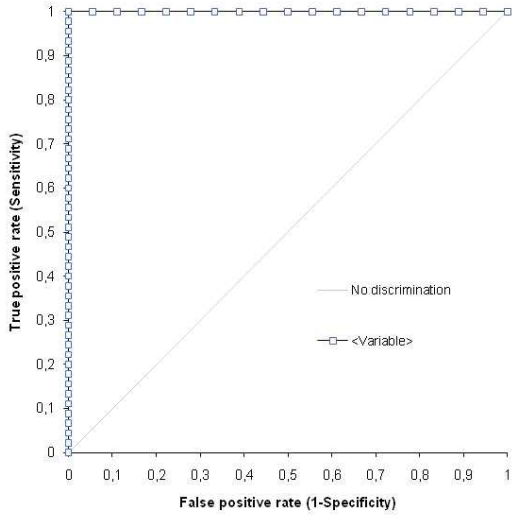
PERFORMANCE DATA

The graph shows the performance Data of AI-LFA MPO using a ROC Analysis in a test Setup using Serumprobes from 22 Vasculitis patients and 45 control sera. The validation test was a commercially available MPO ELISA (Orgentec). For more details please refer to our product information or the respective publication.

n	68

pos	23
neg	45
Prevalence	0,662

Area	95% CI	SE
1,00	0,99 to 1,00	0,003



ROC Analysis MPO AI-LFA vs MPO ELISA

All test results have to be verified with anamnesis and clinical parameters of the tested patient. Sometimes other IVD methods have to be used.

LIMITATIONS OF THE METHOD

The use of haemolytic or lipemic specimens must be avoided. Negative test results cannot rule out a weak immunological reaction of the sample against the tested antigen-

LITERATURE

1. Conrad K, Schößler W, Hiepe F, Fritzler M: **Myeloperoxidase Antibodies.** In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schößler W, Hiepe F, Fritzler M. Pabst; 2007:111-113.
2. Conrad K, Schößler W, Hiepe F, Fritzler M: **Proteinase 3 Antibodies.** In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schößler W, Hiepe F, Fritzler M. Pabst; 2007:147-149.
3. Roggenbuck D, Buettner T, Hoffmann L, Schmechta H, Reinhold D, Conrad K: **High-sensitivity detection of autoantibodies against proteinase-3 by a novel third-generation enzyme-linked immunosorbent assay.** *Ann N Y Acad Sci* 2009, 1173: 41-46.
4. Lucassen R, Schulte-Pelkum J, Petschinka M, Fooke M. **New sensitive and reliable Lateral flow assay for the detection of proteinase 3 and myeloperoxidase antibodies**. Report on the 10th Dresden Symposium on Autoantibodies 2011: 694-695.

PRECAUTIONS FOR USERS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
2. The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents.
3. The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
6. Do not use kit components when the package of the component is damaged. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
7. Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
8. Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
9. The following safety rules should be followed with all reagents:
   - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260).
   - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
   - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
   - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
   - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
   - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
   - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
10. The preservatives (Bronidox L, Azid) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water.
11. Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
12. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

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Lot- Number	European conformity	For *in-vitro* diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manu-factured by	Bio-hazard