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ImmcoStripe™ Vasculitis LIA

Line immunoassay (LIA) for the detection of MPO, PR3, and GBM IgG antibodies

IVD

PRODUCT INSERT

REF 6030 Vasculitis LIA 20 Determinations

INTENDED USE

Line immunoassay for the qualitative detection of MPO, PR3, and GBM IgG antibodies in human serum.

SUMMARY AND EXPLANATION

The presence of anti-neutrophil cytoplasmic antibodies (ANCA) in patients with vasculitis was first observed in 1982 by Davies.[1] ANCA are a group of autoantibodies directed against proteins in the granules of neutrophils. These antibodies can be detected by indirect immunofluorescence on ethanol fixed neutrophils, producing a characteristic staining patterns.[2,3] Two types of ANCA (p and cANCA) are present in patients with small vessel vasculitis. pANCA occur in vasculitis such as glomerulonephritis, Churg-Strauss syndrome, polyarteritis nodosa, systemic lupus erythematosus, and rheumatoid arthritis.[4,5] The major antigen of pANCA is MPO. Additional target antigens such as human leukocyte elastase, and lactoferrin have also been associated with the pANCA fluorescence pattern.[6,7] Antibodies to MPO can also be induced by drugs such as hydralazine, clozapine, and L-tryptophan.[8] Occupational exposure to environmental factors such as silica dust may provoke an anti-MPO positive progressive glomerulonephritis.[8] Measurement of MPO-specific ANCA is an important aid in the evaluation of clinical subtypes within systemic vasculitides.

cANCA are directed primarily against PR3. Some minor antigens that may evoke cANCA reactions are elastase and cathepsin. PR3 is a neutral serine proteinase localized in the azurophilic granules of the neutrophils.[9] Antibodies against the PR3 antigen serve as a marker for Wegener's granulomatosis (WG), a systemic necrotising vasculitis.[10-12] Several studies have established a direct correlation between PR3 antibody levels and the active phase of WG. The concentration of serum anti-PR3 rises dramatically during disease exacerbations (90% frequency), and relapses are usually accompanied by significant titer increases.[13,14] The presence of cANCA is also indicative of other diseases like idiopathic immune necrotizing glomerulonephritis and inflammatory bowel disorders like ulcerative colitis.[15,16]

Rapidly progressive glomerulonephritis (RPGN) is a clinical syndrome developing over days or weeks characterized by crescentic glomerulonephritis on histopathology of the kidney. The prognosis is poor if not recognized early and if an appropriate treatment is not instituted. To optimize patient management, RPGN may be classified based on a) clinical assessment, b) direct immunofluorescence and electron microscopic studies of renal biopsy and c) serum antibody studies. Using the above criteria, RPGN may be classified into a) immune complex mediated disease characterized by the presence of anti-DNA antibodies or anti-streptococcal antibodies, b) anti-glomerular basement membrane (GBM) mediated glomerulonephritis and Goodpasture's Syndrome and c) anti-neutrophil cytoplasmic antibody (ANCA) associated glomerulonephritis. In a study by Jayne et al, of 889 RPGN suspected patients, 47 (5%) had anti-GBM, 246 (28%) had ANCA and 576 (65%) had neither antibodies. Two percent had both ANCA and anti-GBM antibodies.[19] Anti-GBM antibodies can be detected by indirect immunofluorescence or by ELISA or by LIA.[20-28] The antigen associated with anti-GBM antibodies is a non-collagenous domain of collagen IV.

The ImmcoStripe™ Vasculitis LIA offers a convenient method for the identification of MPO, PR3 and GBM antibodies for associated disease specificities.

PRINCIPLES OF PROCEDURE

To perform the test, strips are incubated with diluted patient serum. In positive sera antibodies specifically bind to one or more of the test lines on the strip. The strips are washed according to the protocol, and then the pre-diluted, ready-to-use conjugate is added to the test strips. After incubation and wash steps, the ready-to-use

substrate is added to the strips. During a 10 minute incubation, conjugate and substrate binding produces visible blue/purple lines for serum, conjugate and cut-off control lines. If the sample is positive for any of the antigen coated test lines, it will show a reaction more intense than the cut-off line. Reactions are read visually and reported as positive, negative or equivocal (comparable to cut-off line).

Materials Provided

Vasculitis LIA REF 6030

Kits contain sufficient reagents to perform 20 determinations.

20 x	STRIP VASCULITIS LIA	**Line Immunoassay Test Strips** containing antinuclear antigen coated test lines and control lines. Ready for use.
1 x 120 µl	CONTROL + LIA	**Positive Control** (red cap). Contains human serum positive for one or more markers.
1 x 30 ml	CONJ LIA	**IgG Conjugate**.
1 x 30 ml	SUBSTRATE	**Enzyme Substrate** (amber bottle). Ready for use. **Protect from light**.
1 x 50 ml	DIL	**Diluent**
1 x 50 ml	BUF WASH LIA	**Wash Buffer Concentrate**. **Reconstitute to one liter** with deionized or distilled water or as needed proportionally.
2 x		LIA 10 well Assay Trays
1 x		Report/Scoring Sheet

Symbols used on labels

LOT	Lot number
REF	Catalog number
IVD	In vitro diagnostic use
	Use by
	Storage temperature
	Consult instructions for use
	Number of tests
	Manufacturer
	Date of Manufacture

Materials Required But Not Provided

- Clean 1000 ml graduated cylinder
- Non-serrated forceps (Filter forceps)
- Rocker or rotating platform shaker
- Absorbent paper or paper towels
- Deionized or distilled water
- Squeeze bottles to hold diluted wash buffer or distilled water
- Pipettes capable of delivering 10 to 1000 µl
- Disposable pipette tips
- Timer

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C; **do not freeze.**

All reagents must be brought to room temperature (18-25°C) and mixed thoroughly prior to use. Do not use if reagent is not clear or if insoluble precipitate is present. The reagents are stable until the indicated expiration date when stored at 2-8°C and protected from contamination, or as stated below after opening and/or reconstitution.

- Antigen coated test strips STRIP VASCULITIS LIA are ready for use. Please allow the test strip bag to reach the room temperature before opening to avoid condensation and associated deterioration. Please re-pack unused test strips and store at 2-8°C in dark and dry conditions.
- Sample Diluent DIL is ready to use. After opening, Sample Diluent is stable for at least 8 weeks when stored properly and protected from microbial or chemical contamination.
- Reconstitute 1 part BUF WASH LIA into 19 parts of distilled or deionized water to produce 1 liter of Wash Buffer. Wash Buffer is stable for at least 8 weeks after reconstitution when stored properly and protected from microbial contamination.
- CONJ LIA and SUBSTRATE are stable for at least 8 weeks after opening when stored properly and protected from microbial contamination. SUBSTRATE is light sensitive and must be stored in the provided amber colored bottle.

Antigen strips can only be used once. Do not interchange components of different lots. Do not use reagents beyond expiration date indicated on labels.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of the above materials.[17]

WARNING – Proclin 300 is a preservative. Upon disposal of liquids containing Proclin 300, flush with large volumes of water to dilute the components below active levels.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use kit components beyond expiration date on the labels.

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Specimens with gross hemolysis, elevated lipids or microbial contamination may interfere with the performance of the test and therefore must not be used. Store specimens at 2°-8°C for no longer than one week. For longer storage, serum specimens should be frozen. It is recommended that frozen specimens be tested within one year. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes

- Read Product Insert carefully before starting with the assay.
- Let serum specimens and test reagents equilibrate to room temperature for approximately 30 minutes prior to starting the test procedure. Return all unused specimens and reagents to the refrigerator promptly after use.
- Proper washing technique is critical to the satisfactory performance of the assay.
- Handle test strips with clean forceps or gloves only. Avoid touching the white antigen coated areas.
- The test lines are placed above the cut-off, serum and conjugate control lines as described in the schematic (Figure 1). Serum and conjugate control lines appear on the same piece of nitrocellulose at the bottom of the strip.
- Assign specimen identification numbers to the respective strips on the Report Form. Each strip has the strip number and lot number printed on the bottom for traceability.
- Complete all other relevant information on the Report Form prior to starting the assay.

Test Method

Step 1 Using gloves or blunt forceps, peel off the required number of strips. Care should be taken not to touch the antigen coated areas with bare hands or pointed forceps.

Step 2 Place required number of STRIP VASCULITIS LIA labeled side up into individual wells of the assay tray.

Step 3 Pipet 1.5 ml of DIL into each well; make sure that the strips are completely submerged under the liquid.

Step 4 Incubate the strips in DIL for at least 10 minutes on a rocker. The blue color in coated antigen and control locations starts to disappear as the membrane is soaked.

Step 5 Pipet 15 µl of serum or positive control sample into the appropriate wells to obtain a 1:101 dilution. Incubate 60 minutes (±5 min.) at room temperature on a rocker or rotating shaker.

Step 6 WASH: Aspirate sample solution into waste container. Thoroughly wash strips with the **reconstituted** Wash Buffer by squirting approximately 2ml of solution directly onto strips. Wash strips with gentle agitation for 5 minutes and aspirate solution into waste container. Repeat the wash two more times.

Caution: Complete washing of the strips between incubations is crucial to obtain valid results. Improper washing will result in high background staining. Do not allow the strips to become dry at any step during the assay.

Step 7 Pipet 1.0 ml of CONJ LIA into each well. Incubate 30 minutes (±5 min) at room temperature on rocker or rotating shaker.

Step 8 Repeat Step 6.

Step 9 Pipet 1.0 ml SUBSTRATE TMB into each well and incubate with gentle shaking 10 minutes at room temperature in reduced light. The serum and conjugate control lines develop intense color after incubation in substrate. The cut-off control line develops into a blank to faintly colored line after the incubation.

Step 10 To stop the reaction, rinse strips 2x with distilled/deionized water by squirting approximately 2ml of water directly onto strips followed by aspiration. Do not soak/wash for more than 10 minutes as this may result in decreased sensitivity of the developed colored lines.

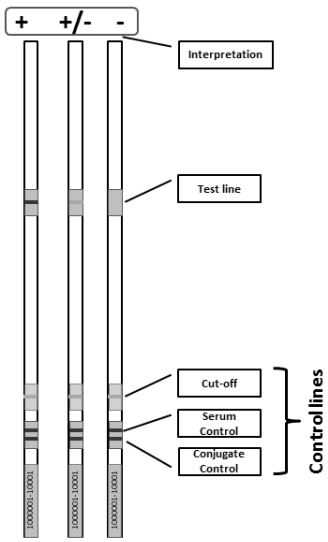
Step 11 Using blunt forceps remove strips from assay tray and place them gently onto absorbent paper and allow them to dry. Let the strips dry before analysis or affixing them on the report/scoring sheet.

Quality Control

Procedural Controls: Each strip has three procedural controls for the addition of serum and conjugate and a cut-off line for determining the weak or negative reactions.
Positive and Negative controls are available as optional components and may be run for additional quality control.
Individual labs are expected to optimize the substrate development time by +/- 4 minutes based on the blot processor or manual methodology. It is recommended that the cut-off line should be faintly visible to the eye, post incubation with substrate.

Interpretation

The test strips contain control lines at the bottom and test line above the controls. The bottom end of the test strip (near serial number) has three control lines: the cut-off line, the serum control line and the conjugate control line from top to bottom. The cut-off allows the technician to determine the test result as positive, negative or indeterminate (+/-). The two procedure control lines ensure the addition of specimen, conjugate and substrate.



Compare the reaction of the test lines with those of the controls. Use of a magnifying glass can assist in observation of weak reactions.

- As labeled in Figure 1, the serum and conjugate control lines should be clearly positive indicating a successful experiment. The cut-off is a faint line with variation in intensity based on the experimental conditions. The schematic in Figure 1 shows an example test line. In the Vasculitis LIA (Figure 2) there are 3 test lines and 3 control lines. The test line development depends on the sample. Positive reactions can occur in varying intensities from weak to strong. **Weak reactions should be compared with intensity of the provided cut-off line within the strip**. Reactions that are distinctly darker or denser than the intensity of the cut-off line should be considered positive.

- Strips may show a homogeneous or discolored background due to various interfering factors in lipemic or hemolytic sera. This effect can also be seen if the test strips are not sufficiently blocked or accidentally allowed to dry up during the assay.
- In case of weak positive and negative reactions, the reacted line intensity should be compared to cut-off line to determine the result as negative (weaker intensity than the cut-off line) or equivocal (+/-; indistinguishable from cut-off line). Visualization of weak reactions is improved when the strips are completely dry. Equivocal samples should be confirmed by an alternate method.
- Dried strips can be assembled in the provided report/scoring sheet. The plastic protective flap is permanently affixed to the report sheet on the left edge. Carefully peel the plastic flap in the right to left direction like a page of the book. Place the reacted strips on the adhesive tape in the respective slot and cover the plastic flap back in place. The protective plastic flap is designed to be reusable for multiple sessions of experiments and the strips can be assembled in respective slots. The technician can use the form to record the lot numbers of used reagents, specimen number and results/comments.

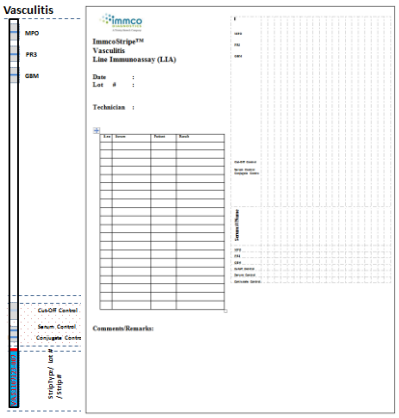


Figure 2: Schematic of Report Sheet

LIMITATIONS OF PROCEDURE

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Do not store specimen at 2-8°C more than a week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

The Vasculitis LIA should only be used as an aid to diagnosis. Positive results may be found in other autoimmune conditions or certain infectious diseases. Hence, results should be evaluated and interpreted by a medical authority in light of the patient's clinical history and other laboratory findings.

EXPECTED VALUES

Incidence of autoantibody positivity varies to a great extent based on the specific antigens, studies and selected cohort of specimens. The expected values in a normal population for Vasculitis antigens are negative on LIA. LIA panels are designed to provide optimal sensitivity and specificity. It is recommended to confirm the positive result by an alternate methodology.

PERFORMANCE CHARACTERISTICS

Antigen Specificities

The Vasculitis LIA is able to detect autoantibodies to the following antigens: MPO, PR3 and GBM. Refer to Figure 2 for individual antigen and control line positions.

Cross-reactivity

A panel of potentially cross-reactive autoimmune disease sera from conditions not associated with Vasculitis was tested using the Vasculitis LIA test. 0 out of 57 determinations demonstrated a positive reaction indicating a specificity of 100% in this population.

Interference

Interference was studied by mixing sera with known levels of autoantibodies for each analyte with potentially interfering serum samples and studying deviation from expected results. No significant interference was demonstrated for the following substances at the levels indicated: Hemoglobin (5 mg/ml), Bilirubin (0.4 mg/ml), Rheumatoid Factor (200 EU equivalent) and Triglycerides (25 mg/ml). Interference studies have been performed according to CLSI guidelines (publication EP7-A2).

Reproducibility

Assays of samples in the negative range, equivocal and positive range were performed to determine qualitative reproducibility from run to run and operator to operator. Results produced 100% qualitative agreement.

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For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: **(716) 691-0091**
Fax: **(716) 691-0466**
Toll Free USA/Canada: **1-800-537-TEST**
E-Mail: info@immco.com

CE

EC REP

EMERGO Europe
Molenstraat 15, 2513 BH, The Hague,
The Netherlands
www.emergogroup.com

or your local product distributor

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