INTENDED USE

The SCIMEDX Corporation Scanlisa enzyme immunoassay (EIA) kit is for the *in vitro* diagnostic detection of anti-Glomerular Basement Membrane (GBM) IgG antibodies in human serum as an aid in the diagnosis of autoimmune renal disorders such as Goodpasture's syndrome.

CLINICAL SIGNIFICANCE

Goodpasture Syndrome is a life threatening autoimmune kidney disease. In 1919 Ernest Goodpasture described coexisting fatal pulmonary hemorrhage and proliferative glomerulonephritis of a young victim of influenza pandemic[1]. Stanton and Tange used the term Goodpasture's Syndrome in 1958 to characterize these manifestations[2]. Goodpasture's Syndrome is now defined as the triad of Glomerulonephritis, Lung Hemorrhage and autoantibodies specific to the non-collagen region (NC1) of the α3 (IV) collagen chain[3] . The Goodpasture's Syndrome is rare and autoantibodies against GBM are not found in normal healthy individuals. Patients in remission or plasmapheresed patients may have a low titer result. It has been indicated that most autoantibodies related to Goodpasture's Syndrome are restricted to NC1 region of the α3 (IV) chain[4]. Patients with Goodpasture's Syndrome often have autoantibodies to other α (IV) chains, but this could be regarded as a secondary response as the disease progresses. Patients with a low titer to the α3 (IV) can have a higher titer to the other α (IV) chains. These patients however have a much milder disease[4,5].

The Glomerular Basement Membrane is composed of many different proteins. Patients with Systemic Lupus Erythematosus, IgA Nephropathy, Post-Streptococcal Glomerulonephritis, Chagas disease, Systemic Small Vessel Vasculitis or other connective tissue diseases, may have antibodies against different GBM antigens[3]. This assay detects primarily antibodies specific to the NC1 region of the α3 (IV) collagen chain. Cross-reactivity with other GBM antibodies has been minimized by purifying the antigen.

Some patients with ANCA or MPO-ANCA antibodies have clinical features suggestive of anti-GBM antibody induced Goodpasture's Syndrome, indicating that serological analysis in reno-pulmonary syndrome should include c-ANCA related tests, MPO-ANCA and anti-GBM[6].

PRINCIPLE OF THE TEST

Diluted sera are incubated in assigned wells and antibodies against GBM antigen present in the specimen bind to the antigen in the wells. Unbound antibodies are rinsed off and a second incubation with goat anti-human IgG conjugated to the enzyme Alk. Phosphatase follows. Unbound conjugate is rinsed off and the bound conjugate is visualized by incubation with pNPP, a substrate that turns yellow when degraded by the enzyme. After the incubation with the enzyme, the reaction is stopped with 1.2M NaOH. The intensity of the color is recorded at 405 nm, and correlates directly with the titer of the anti-GBM in the specimen. The whole assay can be performed in less than 2 hours.

KIT COMPONENTS:

ITEM	CONTENTS	QUANTITY
1.	GBM Antigen coated break apart wells	12 X 8 wells
2.	Goat antihuman IgG – Alk. Phos. Conjugate (Ready to Use)	1 X 15 mL
3.	Calibrators: (Ready to Use) 320, 80, 20, and 5 EU/mL	1ea x 0.75 mL
4.	Positive Control (Ready to Use)	1 x 0.75 mL
5.	Negative Control (Ready to Use)	1 x 0.75 mL
6.	Sample Diluent (Ready To Use)	1 x 75 mL
7.	Wash Buffer (10X)	1 X 100 mL
8.	pNPP Substrate (Ready To Use)	1 X 15 mL
9.	Stop Solution (Ready to Use)	1 X 30 mL

NOTE: Liquid reagents contain the preservative Proclin 300 ™

MATERIALS REQUIRED BUT NOT PROVIDED

- Tube rack in a microplate configuration.
- Multichannel pipettor of 50 - 250 µL range, and micropipettors of 200 - 1000 µL and 2 - 20 µL ranges, and Pipette tips.
- 1 L Graduated cylinder.
- Cover for microplate.
- Clean container for diluted Wash Buffer.
- Reservoirs (disposable).
- Lint free paper towels.
- Calibrated microplate reader adjusted to read at 405 nm.
- Timer (30 min. range).
- Distilled or deionized water.

PRECAUTIONS

1. **Caution**: All blood products should be treated as potentially infectious. Human source materials from which this product was derived were found to be nonreactive for Hepatitis-B surface antigen (HBsAg), HCV, and Human Immunodeficiency Virus 1 & 2 (HIV) antibody when tested in accordance with current FDA required tests. Nevertheless, no known test method can offer total assurance. Therefore, human serum components and patients' specimens should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual - "Biosafety in Microbiological Laboratories", 1984.
2. Reagents in this kit contain 0.1% Proclin® 300 as a preservative.
3. All components in this kit have been tested and standardized as a unit. Do not intermix components from different kit lots or other manufacturer's kits.
4. For *in vitro diagnostic use* only.
5. All reagents must be at room temperature (21-25°C) before running the assay. Temperature WILL affect the absorbances of the assay, but will not change the EU values calculated from the standard curve.
6. Use only distilled or deionized water and clean glassware.
7. Stop Solution should be handled carefully as it can cause burns or irritation to the skin and eyes. If contact occurs, flush immediately with water.
8. Negative and Positive Controls, as well as the Calibrators, must be run with each assay.
9. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
10. Use reservoirs only for single reagents. This especially applies to the substrate reservoir. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn the Substrate Solution yellow.
11. Mix the contents of the microplate wells thoroughly to ensure good test results.
12. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
13. Do not use reagents past their expiration date.
14. Using incubation times and temperatures other than those specified may give erroneous results.
15. Do not reuse microwells and do not pour reagents back into vials as reagent contamination may occur.
16. No assurance is given that these reagents are free of microbial or fungal contamination.

STABILITY AND STORAGE

1. The kits should be stored at 2 - 8°C. Do not freeze.
2. Discoloration of the liquid Substrate ranging from light yellow to intense yellow indicates substrate deterioration and the material should not be used.
3. The Ready to Use Conjugate, Calibrators, and Controls are stable up to the expiration dates listed on the respective bottle labels when stored at 2-8°C. Never freeze the solutions.
4. Diluted 1X Wash Buffer remains stable for 90 days at room temperature.
5. After opening the foil pouch, unused microplate strips coated with the protein antigens should be resealed with the desiccant provided.

SERUM COLLECTION

A whole blood sample should be collected by qualified personnel using approved aseptic venipuncture techniques. Obtain and/or clarify serum samples by centrifugation. The samples may be stored at 2 - 8°C if testing is to be done within 5 days. If stored longer, they should be frozen at -20°C or lower. Do not use a frost-free freezer which may allow the specimens to go through freeze-thaw cycles that can denature the IgG antibody and cause spurious results. Do not use hyperlipemic, hemolytic, heat treated or contaminated samples.

PREPARATION OF REAGENTS AND SAMPLES

1. Bring **all** reagents to room temperature before use. Remove the number of wells being used for the day's testing and replace the remainder in foil pouch with desiccant at 2-8°C.
2. Sample Diluent (Green): "Ready To Use".
3. Calibrators (green): The Calibrators are "Ready to Use" and the assigned values are indicated on their labels. **Do not dilute further**.
4. Positive and Negative Controls: "Ready to Use". **Do not dilute further.**
5. Patient's Sera: Dilute each patient serum 1:50 with Sample Diluent. Prepare dilutions (10 µL + 0.490 mL) in cluster tubes. Mix by inverting the tubes a few times or use a vortex on a low speed.
6. Assign each well the appropriate sample. For improved accuracy, duplicate wells for the Calibrators and Controls may be run**.** Use well A-1 as a reagent blank if a reference wavelength of 620 nm is not available on the reader. This "blank" well should contain assay diluent, conjugate and substrate.
7. Conjugate: "Ready to Use". **Do Not dilute further.**
8. pNPP liquid Substrate: "Ready To Use".
9. Wash Buffer (10X): Dilute the buffer 1 + 9 using distilled or deionized water or by pouring total contents into a graduated cylinder and add distilled or deionized water to 1,000 mL final volume. Mix thoroughly and store the 1X solution at 2 - 8°C.
10. Stop Solution (1.2M NaOH): "Ready To Use".

ASSAY PROCEDURE

1. Transfer 100 µL/well of Sample Diluent into well A-1 for Blank, if appropriate (see # 6 above). Transfer 100µL of Calibrators, Controls and each diluted sample to the corresponding position in the microwell strips. Cover the microwells and incubate for **30** minutes at room temperature.
2. Wash 3 times with 300 µL/well of 1X Wash Buffer. If washing is done manually, empty the plate by shaking into a suitable container and blotting inverted on a paper towel.
3. Dispense 100 µL of Ready to Use Conjugate into each well (including A-1) using a multichannel pipettor. Cover the microwells and incubate for **30** minutes at room temperature.
4. Wash as described above in step # 2.

5. Add 100 µL of Substrate Solution to all wells using a multichannel pipettor. Cover the microwells and incubate at room temperature for **30** minutes.
6. Terminate the reaction by adding 100 µL of Stop Solution (1.2 M NaOH) to each well using a multichannel pipettor. Shake the plate for 3-10 seconds to mix. Be careful not to splash the solution from the wells. The wells must be read within 30 minutes after stopping the reaction.
7. Results: Zero the reader on air and read the absorbance of the wells at 405 nm **or** use the reference wavelength of 620 nm in an appropriate microplate reader.
   NOTE: The wavelength of microplate or strip reader used should be set at 405 nm. If a reference wavelength is not available zero header on air, alternatively use a reagent blank consisting of diluent, conjugate, and substrate. The absorbance of the reagent blank (A-1) is automatically subtracted from each well before calculations are done. The absorbance at 405 nm of the samples, Controls and the Calibrators must always be determined at the same time.

CALCULATION OF RESULTS

Calibrators:

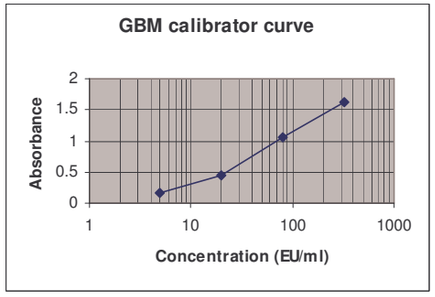
1. A curve is constructed from the concentration of the Calibrators versus the Absorbances. These values are used to calculate the EU/ml of antibody in the test specimen.
2. Graph Method:
   A. Plot the absorbance values for the Calibrators on the linear ordinate (Y axis) against the corresponding EU/ml on the logarithmic abscissa (X-axis) using log/lin graph paper.

   B. Locate the point corresponding to the absorbance value (Y-axis) of each specimen and read its corresponding EU/ml from the X-axis.
3. EIA Software Program:
   Using an EIA reader with the appropriate software for data reduction, choose log/lin for X/Y axis and point-to-point or equivalent as your choice for a curve fitting program.
   NOTE: Assigned values for the calibrators are expressed as EU/ml and are located on the label.

Example: Using log/lin graph paper, the following standard curve is plotted:

From the assay the absorbance of the Positive Control is: **0.985**

1. The absorbance is found on the Y-axis;



2. The line is followed to the standard curve;
3. From the curve the line is followed to the X-axis to locate the EU/mL for the sample.
4. The value for the Control in this example is: **67 EU/mL**

4. Dilution of Serum
   Samples which give absorbances above the top Calibrator (320 EU/mL) are out of the range of this assay, and should be stated as >320 EU/mL. Such samples may be diluted as appropriate.
   A further dilution of 1:5 is recommended. When calculating the

results for a diluted sample, the concentration obtained must be corrected by the dilution factor (X 5).

INTERPRETATION OF RESULTS

Antibody EU/mL values are reported as a decimal number (30.4 EU/mL). The numeric value should be used.

Positive = Equal to or greater than 15 EU/mL

Equivocal = 5.1 – 14.9 EU/mL

Negative = Equal to or less than 5 EU/mL

If an equivocal result is obtained, re-test the sample. If upon repeat, the sample remains equivocal, it can be assumed that there is not a significant level of antibodies present in the sample to be classified as definitive positive. The sample should be reported as equivocal and evaluated by the physician in the context of the patient's overall history

QUALITY CONTROL

Positive and Negative Controls are supplied with the kit. The controls verify test performance, test integrity and operator reliability. Good laboratory practice dictates running the positive and negative control each time the kit is used. If a result within the confidence limit (as listed on the label) of the positive and negative control is not obtained, test results are not valid.

If a repeat assay is performed, calibrators and controls must also be run in the assay. Always use fresh dilutions of the patient samples, regardless of when the dilutions were made.

If sufficient results cannot be obtained, contact the SCIMEDX Technical Service Department.

LIMITATIONS

1. The antibody titer obtained from individual samples does not necessarily correlate with disease severity and should not be reported as such. Antibodies from different patients may have different avidities.
2. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures. Whenever possible, the pathology of a renal biopsy should be obtained.
3. Use fresh serum or samples frozen only once and thawed. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
4. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as rinsing the test strips and thorough mixing of all prepared solutions.
5. Do not scratch coated wells during washing and aspiration. Dispense and fill all reagents without interruption. While dispensing, check that all wells are filled evenly with Washing solution and that there are no residues in the wells.
6. Instructions for using appropriate photometers are to be observed; check adjustment of proper wavelength and reference wavelength respectively.
7. Use fresh serum or samples frozen only once and thawed. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
8. Proper washing of the microwells is critical for reducing the potential of nonspecific reactions or residues causing false positive reactions. The use of automated washing equipment may require 4-5 rinses for each washing step.

EXPECTED RESULTS

Anti-GBM antibodies are not found in normal healthy individuals. Of 500 new renal disease patients per million people per year, Goodpasture's

syndrome is expected in no more than 0.5%. It is expected that all untreated Goodpasture's syndrome patients will test positive for anti-GBM antibodies. Published data has demonstrated the potential incidence of GBM antibodies in patient serum with histologically verified glomerulonephritis, renal vasculitis, SLE, and other connective tissue diseases.[6] Another study[7] included evaluation of patients, which were known positive for ANA, Anti-DNA, RF, ASMA , and AMA. The following table summarizes the results of these studies:

Diagnosis	Number Tested	No. GBM positives
Anti-nuclear Antibody	10	0
Anti-DNA	5	0
Rheumatoid Factor	10	1
Anti-Smooth Muscle Antibodies	4	0
Anti-Mitochondrial Antibodies	4	0
Systemic Small Vessel Vasculitis	64	3
Goodpasture's Syndrome	14	14
Henoch-Schonlein purpura	20	1
Systemic Lupus Erythematosus	44	5
Other Connective Tissue Diseases	43	1

PERFORMANCE CHARACTERISTICS

Comparison

A Study was performed using 166 patient samples that were characterized as GBM positive or negative and 216 normal human serum acquired from a blood bank. In addition 79 potential cross reactive auto immune samples were tested. These samples were assayed in the Scanlisa GBM kit and the results compared to the reference ELISA GBM kit. In the Scanlisa assay samples are regarded as negative when equal to 5 or below 5 EU and positive if equal to or above 15 EU. The 5.1-14.9 EU range is considered borderline and a sample should be re-tested. The results yielded a sensitivity of 98.6% for positive samples, a specificity of 100% for normal human sera and a 95.8 % specificity for auto immune samples, when equivocal samples were excluded. The agreement with the reference ELISA was 98.7% for positive samples, 99.5% for normal human serum samples, 93.7% for auto immune.

Relative Agreement between the Reference Kit and the Scanlisa Kit = (437/461) 97.3%.

Postive and Negative samples	Reference GBM ELISA	Scanlisa GBM
Postive	160	145
Equivocals	N/A	12
Negative	6	9
Total	166	166

Scanlisa Relative Sensitivity = (145/147) 98.6% (excluding equivocals). 15 samples were discrepant with the reference ELISA when equivocal were classified as negative. These samples were tested in a 510K approved IFA. Thirteen of these samples agreed with the Scanlisa in classifying them as negative. Two samples were determined to be positive in IFA and the reference ELISA, but only equivocal in the Scanlisa assay.

216 normal blood bank donor samples were used to evaluate the value range of this population with the Scanlisa GBM EIA kit. The mean value of the concentration of these normal patients was 3.1 EU/ml with a standard deviation of 0.5

Normal human serum samples	Reference GBM ELISA	Scanlisa GBM
Postive	3	0
Equivocals	N/A	2
Negative	213	214
Total	216	216

Scanlisa Relative Specificity = (214/214) 100% (excluding equivocals)

Cross Reactivity

A study was performed using 79 positive autoimmune disease characterized samples obtained from an outside laboratory. The samples

were run on the Scanlisa kit and a reference EIA kit. Three of the disease states tested, showed a false positive reaction in the Scanlisa GBM IgG Antibody detection kit. These three samples were from patients with SLE or Mixed Connective Tissue Disease. This is consistent published literature[6]. Two samples cross-reacted with the reference ELISA. This study demonstrates that diseases such as myopathy, dermatitis, sclerosis, polymyositis, rheumatoid arthritis, do not interfere with the assay, but SLE and Mixed Connective Tissue Disease samples may occasionally interfere in this assay.

Auto immune samples	Reference GBM ELISA	Scanlisa GBM
Postive	2	3
Equivocals	N/A	8
Negative	77	68
Total	79	79

Reproducibility

Five sera (4 positive and 1 negative) were run in 16 replicates for **Intra-Run** reproducibility. The following EU/mL results were obtained:

	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
MEAN	262.0	114.6	42.3	9.7	2.8
S.D.	16.4	6.5	1.8	0.3	0.1
% C.V.	6.3	5.7	4.3	3.5	4.2

Five sera (4 positive and 1 negative) were run in triplicate on 3 lots of GBM kits to evaluate the **Inter-Lot** reproducibility. The following EU/mL results were obtained:

	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
MEAN	326.3	150.5	70.3	13.9	2.2
S.D.	45.4	14.3	8.0	1.4	0.3
% C.V.	13.9	9.5	11.4	10.2	13.2

Five sera (4 positive and 1 negative) were run in duplicate over 5 separate days to evaluate the **Inter-Day** reproducibility. The following EU/mL results were obtained:

	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
MEAN	295.1	72.2	53.4	10.4	3.5
S.D.	13.3	10.8	3.0	0.8	0.3
%C.V.	4.5	14.9	5.7	8.1	9.6

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