

Introduction:

Many different types of reticulin antibodies have been detected by immunofluorescence. Two types of fibrillar antigens can be detected by staining patterns, anatomical distribution and species specificity. A third antibody reacts with the mucopolysaccharides lining the hepatic sinusoid (ground substance antigens), small fibers, and amorphous proteins. In addition, intrasinusoidal cells, such as Kupffer cells and glass-adherent blood-borne cells, exhibit cytoplasmic fluorescence. These antibodies related to the reticulin antigens are most frequently associated with Celiac Disease and Dermatitis Herpetiformis. The antibodies appear to be stimulated by bacterial or nutritional antigens.¹

The use of rat liver/kidney/stomach tissue can be utilized to screen for reticulin antibodies by immunofluorescence. There are a variety of staining patterns associated with the mesenchymal structures in the liver, kidney and other organs.¹ Many sera appear to contain a mixture of antibodies and stain cells, ground substances and fibers in various combinations. These antibodies may appear individually, and attempts have been made to access their occurrence as they relate to specific diseases.

Two patterns that can be observed in the connective tissue fibers are referred to as reticulin 1 (R1) and reticulin 2 (R2). R1 is related to Celiac's Disease, the staining pattern on a liver substrate exhibits nodular fluorescence around the portal tracts, up to the limiting plate of the hepatocyte, and a fine outline of the sinusoid. R1 sera in the kidney stains all around the tubules and the Bowman's capsule. On smooth or striated muscle tissue there is a fluorescent "honeycomb" appearance. These antibodies are not species specific.

R2 antibodies are species specific and only react with rat tissue. R2 sera stains the thin sharp-edged fibers in the liver. In the stomach (longitudinal section), they appear as streaks between the gastric glands. These fibers are not found in great quantity in the muscularis mucosae but rather they are concentrated around the vessels in all organs. In the kidney, there is no staining of the "Bowman's Capsule" or between the tubules. Only the perivascular connective tissue stains in the liver and stomach. The staining can be mistaken for smooth muscle; care should be taken to

evaluate closely. The R2 fibers are very thin and much sharper than smooth muscle fibers.

Kupffer Cells fluorescence (KC): Fluorescence is observed in isolated large sinusoidal cells and are of irregular shape and size, distributed randomly throughout the liver.

Sinusoidal Adherent Cell (AC): This reaction is observed in a limited number of cases, and the fluorescence is confined to the cytoplasm of small round cells, irregularly distributed throughout the liver. These cells can also be observed in the stomach, ileum and colon.

Reticulin Sinusoidal fluorescence (RS): This pattern shows a diffuse staining of the sinusoid associated with cytoplasmic staining of the Kupffer Cells and other reticuloendothelial cells.

Testing for endomysial antibodies (IgA-EmA), along with anti-reticulin antibodies, should be considered. Anti-endomysial (IgA) antibodies are associated with active CD (Celiac Disease). These antibodies can also act as a monitoring tool for compliance with diet in established CD cases. In addition, the screening for antibody levels has been reported in the scientific journals, to aid in the proper timing of biopsies in patients undergoing evaluation.^{2, 4}

Principles:

The primary test reaction involves circulating anti-reticulin antibodies present in the patient's serum, which bind to the substrate to form an antigen/antibody reaction. This occurs during an incubation period while the serum covers the antigen substrate surface. The secondary reaction utilizes a fluorescein labelled anti-human globulin conjugate that labels the primary antigen/ antibody reaction and can be visualized through the microscope.

Materials Provided:

Storage & Stability of Components:

1. FITC (IgG) Conjugate No. 1528EL (3.0 ml) with Evans Blue Counterstain is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
2. The antigen slides of rat liver, kidney, stomach sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. Reticulin (IgG) positive control No. 5702L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted Buffer does not contain preservatives and should be stored at 2-8 C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.
7. Absorbent Blotters.

Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (1000 ml)
Distilled H₂O
Fluorescent Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20 C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thimerosal or 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:10 in PBS. **Titration:** set up doubling dilutions of serum starting at 1:10, 1:20, 1:40, 1:80, etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30 μ l) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
7. Deliver 1 drop (20-30 μ l) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows

bright fluorescence, either the control, antigen, conjugate or technique may be at fault.

- The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
- In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

Results:

The slide should be examined under 400X, high dry objective. Reticulin-positive result is observed as a 1 + or greater fluorescent reaction of 1 or more of the 5 basic reaction patterns, seen individually or in various combinations.

Pattern Interpretation:

There are at least 5 different antibody patterns that can be observed;

- Reticulin (R1):**
Liver: Nodular fluorescence in the connective tissue space around the portal tract, soft-edge fibers spread into the sinusoid.
Kidney: Soft-edge fluorescence around the renal tubules. Perivascular connective tissue in small arteries.
Stomach: A "Honey-comb" fluorescence.
- Reticulin (R2):**
Liver: Sharp thin fibrils at the edge of the portal tract only.
Kidney: Perivascular connective tissue staining only.
Stomach: Fluorescent long streaks between the gastric gland.
- Kupffer Cell (KC):** Isolated large sinusoidal cells of irregular shape and size, randomly distributed throughout the liver, endothelial cells, connective tissue fibers and portal tracts are negative.
- Adherent Cells (AC):** Cytoplasmic fluorescent reaction of small round cells irregularly

distributed throughout the liver and occasionally seen in the stomach, ileum and colon.

- Reticulin Sinusoidal (RS):** Diffuse staining of the sinusoid associated with the cytoplasmic staining of the Kupffer and other reticuloendothelial cells.

Limitations of Procedure:

- No diagnosis should be based upon a single test result, since various host factors must be taken into consideration.
- Various autoimmune processes, such as SMA(+), can be confused with reticulin antibody. Care should be taken to differentiate the two.

Precautions:

- All human components have been tested by radioimmunoassay for (HB_sAg) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sAg or HTLVIII/LAV. All human components should be handled with appropriate care.
- The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
- Do not use components beyond their expiration date.
- Follow the procedural instructions exactly as they appear in this insert to insure valid results.
- Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
- Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

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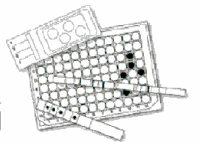
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