

# NOVA Lite® Rat Liver, Kidney, Stomach

704170

For *In Vitro* Diagnostic Use

Product Code: 704170, 704180  
504170.10, 504180.25

CLIA Complexity: High

## Intended Use

This product is intended for use in the screening and titration of circulating autoantibodies in human serum as an aid in the diagnosis and treatment of various autoimmune diseases. The four major autoantibodies detected are antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), antismooth muscle antibodies (ASMA) and antigastric parietal cell antibodies (AGPCA).

## Summary and Explanation of the test

Indirect immunofluorescence is the reference method for screening and titration of circulating autoantibodies in human serum. Animal tissue sections, e.g. from rat, are generally preferred over other commonly-used substrates including human tissue sections and cell preparations; this is primarily due to the lack of interference from HLA and/or other blood group antibodies. Using three different tissues (liver, kidney and stomach) enables autoantibodies to be more easily identified by comparing the results obtained with each tissue.

Particular autoantibodies are associated with a number of different diseases. ANA almost always occur with systemic lupus erythematosus (SLE) but are also commonly found in patients with connective tissue and rheumatoid diseases. AMA are frequently present in primary biliary cirrhosis but may also be detected in patients with other liver diseases. ASMA are frequently associated with chronic active hepatitis and primary biliary cirrhosis, but are also detectable in low concentrations in various other conditions. AGPCA occur in the serum of most patients with pernicious anemia. A more detailed description of which autoantibodies are associated with which disease is given in the Results section.<sup>1-9</sup>

## Principles of the Procedure

An indirect immunofluorescence technique is utilized where patient samples and appropriate controls are incubated with the substrate slides.<sup>10</sup> The unreacted antibodies are washed off and an appropriate fluorescein labeled conjugate is applied. Unbound conjugate is washed off, and slides are viewed with a fluorescence microscope. Positive samples produce apple-green fluorescence which corresponds to areas of the section where autoantibody has bound.

## Reagents

1. Rat liver, kidney, stomach sections on 5 or 10 well slides with desiccant

### Kits only:

2. ANA Homogeneous Pattern, positive control serum containing 0.09% sodium azide. **Prediluted, ready for use.**
3. IFA System Negative control serum containing 0.09% sodium azide. **Prediluted, ready for use.**
4. Affinity purified sheep anti-human IgG (H+L) fluorescein isothiocyanate (FITC) conjugate containing 0.09% sodium azide. **Prediluted, ready for use.**
5. Mitochondrial (AMA) Pattern, positive control serum, containing 0.09% sodium azide. **Prediluted, ready for use.**
6. Smooth Muscle (ASMA) Pattern, positive control serum, containing 0.09% sodium azide. **Prediluted, ready for use.**
7. 1% Evans Blue, Optional counterstain
8. Phosphate buffered saline (PBS), a 40-fold concentrate in liquid form
9. Blotters, to blot the slides after washing
10. Mounting medium, containing an anti-fading agent (DABCO) 1, 4 diazabicyclo [2.2.2] octane
11. Coverslips

## Warnings/Precautions

All donors of human serum supplied (kits only) have been tested and found to be negative for Hepatitis B surface antigen and antibodies to Hepatitis C virus and Human Immunodeficiency Virus (HIV 1 & 2). However, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material and only personnel adequately trained in such methods should be permitted to perform the procedures.

The Evans Blue and the kit controls contain 0.09% sodium azide as a preservative and must be handled with caution – do not ingest or allow contact with the skin or mucous membranes. If contact does occur wash with a large volume of water and seek medical advice. Explosive metal azides may be formed with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up. This product should only be used by suitably trained persons for the purposes stated. Adherence to the given procedure is recommended

## Storage Conditions

Unopened kits/slides should be stored at 2-8°C and can be used until the given expiry date. **DO NOT FREEZE.** Once slides are removed from their foil bag, they should be used immediately. Diluted PBS buffer can be stored for up to one month at 2-8°C. All reagents should be stored at 2-8°C.

## Specimen Collection

Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 7 days prior to assay<sup>11</sup> or for prolonged storage, aliquoted and stored at -20°C or below. DO NOT freeze and thaw sera more than once. Avoid using lipaemic, haemolysed or microbially contaminated sera as decreased titres or unclear staining patterns may occur.

## Procedure

### Materials provided (kits)

#### 704170

1. 10 x *Rat liver, kidney, stomach slide (5-well)*
2. 1 x 1mL *ANA Homogeneous Pattern* (prediluted)
3. 1 x 1mL *IFA System Negative Control* (prediluted)
4. 1 x 1mL *Mitochondrial (AMA) Pattern* (prediluted)
5. 1 x 1mL *Smooth Muscle (ASMA) Pattern* (prediluted)
6. 1 x 7mL *FITC IgG (H&L) Conjugate*
7. 1 x 3mL *1% Evans Blue Counterstain*
8. 2 x 25mL *PBS Buffer (x40 conc)*
9. 1 x 3mL *Mounting Medium*
10. 20 x *Blotters*
11. 10 x *Coverslips*
12. 1 x instruction leaflet

#### 704180

1. 25 x *Rat liver, kidney, stomach slide (10-well)*
2. 1 x 1mL *ANA Homogeneous Pattern* (prediluted)
3. 1 x 1mL *IFA System Negative Control* (prediluted)
4. 1 x 1mL *Mitochondrial (AMA) Pattern* (prediluted)
5. 1 x 1mL *Smooth Muscle (ASMA) Pattern* (prediluted)
6. 1 x 15mL *FITC IgG (H&L) Conjugate*
7. 1 x 3mL *1% Evans Blue Counterstain*
8. 2 x 25 *PBS Buffer (x40 conc)*
9. 1 x 10mL *Mounting Medium*
10. 50 x *Blotters*
11. 25 x *Coverslips*
12. 1 x instruction leaflet

### Materials provided (slides)

1. **504170.10** 10 x Rat Liver, Kidney, Stomach Slide (5 well)  
**Or**
2. **504180.25** 25 x Rat Liver, Kidney, Stomach Slide (10 well)
3. 1 - Instruction leaflet

### Additional Materials Required But Not Provided

Distilled water to dilute PBS concentrate

Container for PBS buffer

Micropipettes and disposable tips to apply patient samples

Humid chamber for incubation steps

Fluorescence microscope with 495nm exciter filter and 515nm barrier filter

Plastic squeeze bottle for initial wash in PBS

Additional components may be obtained from INOVA Diagnostics: PBS (508002), IFA System Negative Control (508186), ANA homogeneous pattern (508101), Mitochondrial (AMA) Pattern (504052), Smooth Muscle (ASMA) Pattern (504053), FITC IgG (H&L) Conjugate (504021, 504072), 1% Evan's Blue (504049) and Mounting Medium (504046, 504047).

## Test Procedure

### Quality control

Positive and negative controls should be used every time samples are tested.

1. **Mounting medium.** Remove the mounting medium from the fridge to allow it to reach room temperature (18-28°C) before it is needed.
2. **Dilute PBS concentrate.** Dilute PBS concentrate with distilled water (1 part PBS concentrate + 39 parts distilled water) and mix. The PBS is used for diluting patient samples and as a wash buffer.
3. **Dilute patient samples.**  
**Screening:** Dilute patient samples 1/20 by adding 50µL of serum to 950µL of PBS buffer.  
**Titration:** Make serial dilutions of positive samples with PBS buffer. (e.g. 1/20, 1/40, 1/80, 1/160 and 1/320, etc).  
For example: Take 100µL of the 1/20 dilution, mix with 100µL PBS to give a 1/40 dilution. Repeat for further dilutions.
4. **Substrate slides.** Allow substrate slide(s) to reach room temperature (18-28°C) prior to removal from pouch(es). Label slides appropriately, place in the humid chamber and add one drop of each positive and negative control to appropriate wells. Add 50µL of diluted patient samples to the remaining wells.
5. **Slide incubation.** Incubate slides for 20 minutes in a humid chamber at room temperature (18-28°C).
6. **PBS Wash.** Remove slides from humid chamber and rinse briefly with PBS squeeze bottle. Do not squirt directly on to the wells. Place slides in a rack and immerse in PBS and agitate or stir for 5-10 minutes.

7. **Addition of fluorescent conjugate.** Shake off excess PBS and blot around wells using blotters provided. Return slides to humid chamber and **immediately** cover each well with a drop of fluorescent conjugate. **DO NOT LEAVE WELLS UNCOVERED FOR LONGER THAN 15 SECONDS.** Drying out of the substrate seriously affects the results.
8. **Slide Incubation.** Incubate slides for 20 minutes in humid chamber at room temperature (18-28°C) in the dark.
9. **PBS Wash.** Wash again as described above. Optional counterstain. Add 2-3 drops of 1% Evans Blue per 100mL of PBS prior to slide immersion.
10. **Mounting with coverslip.** Remove one slide at a time from PBS wash. Quickly dry around the wells and add a drop of mounting medium to each well. Carefully lower slide onto the coverslip, avoiding air bubbles, but if present do not attempt to remove. Wipe excess medium from around edge of coverslip.
11. **View slides under fluorescence microscope.** Slides may be stored for up to 3 days at 2-8°C, in the dark, without significant loss of fluorescence.

## Results

### Quality Control

The ANA Homogeneous Pattern should give a bright apple-green homogeneous pattern in cell nuclei. The Mitochondrial (AMA) Pattern should give bright apple-green staining of renal tubules and gastric parietal cells. The Smooth Muscle (ASMA) Pattern should give bright apple-green staining of the muscularis layer of the stomach. The negative control should show dull green staining in all tissues, with no discernible fluorescence. If the controls do not appear as described, the test is invalid and should be repeated.

## Interpretation of Results

### Negative result

These are seen as dull green staining in all sections, with no discernible fluorescence. Weak reactions should be repeated but at a higher dilution (e.g. 1/40). If the repeated result appears the same as the original, the test is regarded as negative.

### Positive result

These are seen as significant fluorescence in specific tissue areas giving one or more of the following patterns:

#### Antinuclear antibodies (ANA)

ANA stain the nuclei of the following: liver cells, distal and proximal kidney tubules, gastric parietal and chief cells. Almost all SLE patients have ANA in the serum, but ANA can also be found in various connective tissue diseases including rheumatoid arthritis. ANA can also occur with chronic active hepatitis and primary biliary cirrhosis and in response to certain drug treatments.

Additional information can be gained by interpreting the ANA nuclear patterns obtained (see below). It is recommended that all positive ANA samples are titred to endpoint to ensure that possible mixed reactions are detected that may otherwise have been missed. Further analysis of such samples for autoantibodies to double stranded DNA and extractable nuclear antigens (ENA) is also advised.

Pattern	Common antigens involved	Disease association
Homogeneous	ds DNA, histones	SLE Rheumatoid arthritis (RA) Mixed connective tissue disease Drug induced lupus
Peripheral (Rim)	Native/ds DNA	SLE
Speckled	Extractable nuclear antigens, Ribonucleoprotein, Scl-70, SSB	Scleroderma Sjögren's syndrome Mixed connective tissue disease SLE
Nucleolar	4-6S rRNA	Scleroderma Sjögren's syndrome SLE, RA and progressive systemic sclerosis

#### Tissue specific autoantibody staining patterns include:

Antibody	Tissue associated	Main disease association
Antimitochondrial antibodies (AMA)	Kidney distal tubule cytoplasm and the proximal tubules (to a lesser extent). Hepatic cell cytoplasm (liver) and gastric parietal cell cytoplasm (stomach)	Primary biliary cirrhosis & other liver disease
Antismooth muscle antibodies (ASMA)	Muscularis layers (stomach) Arteriole muscle layers (other tissues)	Chronic active hepatitis
Antigastric parietal cell antibodies (AGPCA)	Parietal cells (stomach)	Pernicious anaemia
Antireticulin antibodies (ARA)	Peritubular fibres & Bowman's capsules (kidney) Hepatic cell membranes, sinusoidal walls (liver)	Crohn's disease Coeliac disease <i>Dermatitis herpetiformis</i>

NB: Each laboratory should establish at which point a positive result is considered clinically significant.

## Limitations of the Procedure

1. This test alone should not be considered diagnostic. All other factors including the clinical history of the patients and other serological or biopsy results must also be taken into account.
2. Staining patterns often change as a sample is titred out to end point. This is usually due to the presence of more than one autoantibody, especially for ANAs.
3. A negative result with this kit can be due to disease remission or to the presence of autoantibodies not detectable by this technique.
4. The light source, filters and optics of different makes of fluorescence microscopes will influence the sensitivity of the assay. The performance of the microscope is significantly influenced by correct maintenance especially centering of the mercury vapor lamp and changing of the lamp after the recommended period of time.
5. Autoantibodies can be activated or induced by a wide range of drugs including oral contraceptives and antibiotics.
6. Due to the short distance between wells on the 10 well slides, it is possible for cross contamination of samples and controls to occur. Care must be taken, especially at the washing stages, to ensure that this does not happen.
7. Suitability for use with other manufacturers' IFA reagents has not been assessed but use with such reagents should not necessarily be excluded.

Slides sold separately are classified as "Analyte Specific Reagents". Except as a component of the kit, analytical and performance characteristics are not established.

## Expected Values and Specific Performance Characteristics

### Expected results

#### Normals

Antibody	Occurrence percentage	Reference
ANA	0-2	2
AMA	0-8	7
ASMA	3	2
ARA	5	8
AGPCA	10-15 (in elderly)	9

#### Abnormals

	Clinical Diagnosis	Occurrence percentage	Reference
ANA	SLE	99	4
	Mixed connective tissue disease	90	3
	Other autoimmune disease (RA, scleroderma, Sjögren's syndrome, dermatomyositis)	15-50	3,5
AMA	Primary biliary cirrhosis	>95	3
ASMA	Chronic hepatitis	70	3
	Primary biliary cirrhosis	50	3
ARA	Crohn's disease	24	6
	Coeliac disease	38-50	3,6
	Dermatitis herpetiformis	22	6
AGPCA	Pemicious anaemia	90	9

### Comparison study

A comparison study was performed on 96 clinical samples using this kit and a commercially-available reference method. 93 out of the 96 samples tested gave identical results by the two methods. For the patterns described in the Results section, the relative sensitivity ranged from 89-100%, the relative specificity was 100% in every case and the relative agreement ranged from 93-100%. For the samples that differed, weak ANA or SMA positive staining was obtained in each case by the reference method only, suggesting that these samples were borderline for the particular pattern, and/or there are slight differences in sensitivity between the two methods.

### Summary of the Procedure

1. Equilibrate mounting medium to room temperature.
2. Dilute PBS with distilled water.
3. Dilute patient sera 1/20 with PBS.
4. Equilibrate substrate slides to room temperature (18-28°C).
5. Apply 50µL positive and negative controls and diluted patient sera to appropriate wells.
6. Incubate in a humid chamber for 20 minutes.
7. Wash for 5-10 minutes in PBS.
8. Blot around each well and immediately cover each well with a drop of conjugate.
9. Incubate as in step 6.
10. Wash as step 7.
11. Mount.
12. View slide under fluorescence microscope.

## References

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