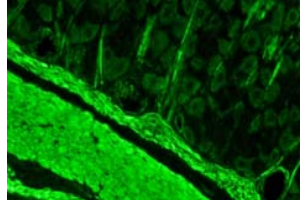
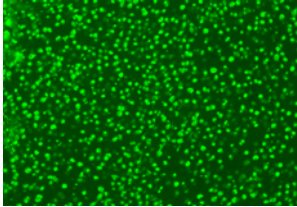


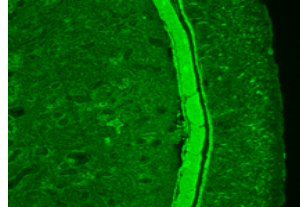
AMA on Mouse Kidney/Stomach



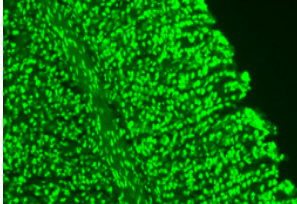
ASMA on Mouse Stomach



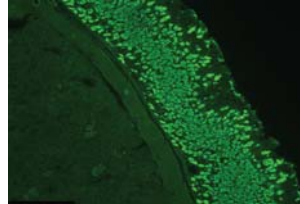
ANA on Mouse Liver



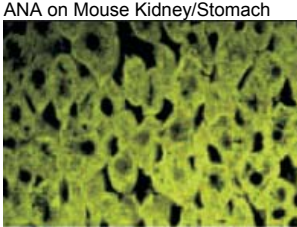
ASMA on Mouse Kidney/Stomach



ANA on Mouse Kidney/Stomach



AGPA on Mouse Kidney/Stomach



LKM on Liver



## Cambridge Life Sciences Ltd. Instructions for Using Immco Slides: ImmuGlo™ Autoantibody Test Mouse Kidney/Stomach/Liver Substrate

**IVD** For *in vitro* diagnostic use

<b>REF</b>	Code: 2152	8 Well Substrate Slide	8 Tests
<b>REF</b>	Code: 2152-1	10 Well Substrate Slide	10 Tests
<b>REF</b>	Code: 2152-3	8 Well Substrate Slide	8 Tests
<b>REF</b>	Code: 2152-10	10 Well Substrate Slide	10 Tests
<b>REF</b>	Code: 2153	8 Well Substrate Slide	8 Tests
<b>REF</b>	Code: 2167	6 Well Substrate Slide	6 Tests
<b>REF</b>	Code: 2167-8	8 Well Substrate Slide	8 Tests
<b>REF</b>	Code: 2169	6 Well Substrate Slide	6 Tests
<b>REF</b>	Code: 2190	10 Well Substrate Slide	10 Tests
<b>REF</b>	Code: 2190LKM	8 Well Substrate Slide	8 Tests
<b>REF</b>	Code: 2191	10 Well Substrate Slide	10 Tests

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Cambridge Life Sciences Ltd  
14 St. Thomas' Place  
Cambridgeshire Business Park  
Ely, Cambs. CB7 4EX UK

Tel: +44(0)1353 645200 Fax: +44(0)1353 645250  
email: sales@clsdiagnostics.com  
www.clsdiagnostics.com

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## INTENDED USE

Indirect immunofluorescence (IF) antibody tests for the detection and quantitation of antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), anti-gastric parietal cell antibodies (AGPA) and anti-liver/kidney/microsomal (LKM) antibodies in human serum.

## SUMMARY AND EXPLANATION

**Antinuclear antibodies (ANA)**, detected by indirect immunofluorescence, aid in the diagnosis of connective tissue disorders including systemic lupus erythematosus (SLE), mixed connective tissue disease, Sjögren's syndrome and scleroderma<sup>1-5</sup>. ANA occur in about 95% of SLE patients as well as patients with other connective tissue diseases. ANA may also occur in other disorders such as chronic active hepatitis and primary biliary cirrhosis<sup>6-8</sup>.

**Anti-mitochondrial antibodies (AMA)** occur in over 90% of primary biliary cirrhosis cases, 3-11% of chronic active hepatitis patients and are absent in patients with extrahepatic biliary obstruction and in other liver diseases. The universal presence of anti-mitochondrial antibodies in primary biliary cirrhosis and their virtual absence in extrahepatic jaundice makes their detection of considerable value in the differential diagnosis<sup>6-12</sup>.

**Anti-smooth muscle antibodies (ASMA)** in high titre (>160) occur in the majority of cases of chronic active hepatitis and in intermediate titres (40-80) in acute viral hepatitis. Occasionally they may occur in cases of primary biliary cirrhosis where they are also found in intermediate titres. The significance of titres of 20-40 is doubtful since these titres may occur in normal individuals<sup>13, 14</sup>.

**Anti-gastric parietal cell antibodies (AGPA)** are commonly associated with pernicious anaemia and chronic atrophic gastritis where they occur in about 90% and 50% of cases, respectively. However, they are not disease specific as they may occur in low frequency in other disorders. Although healthy individuals may have gastric parietal cell antibodies, this finding may reflect asymptomatic atrophic gastritis. Negative findings for gastric parietal cell antibodies provide strong evidence for excluding pernicious anaemia<sup>15-17</sup>.

**Anti-liver/kidney/microsomal antibodies (LKM)** are microsomal antibodies that exhibit characteristic reaction staining of the cytoplasm of liver and proximal tubules of the kidney tissue. They are found in a group of patients with autoimmune hepatitis<sup>18-20</sup>. LKM antibodies are primarily of the IgG isotype and can be differentiated by various reaction patterns.

## PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method used, patients' sera are incubated on mouse kidney/stomach/liver sections to allow binding of antibodies to the substrate. Any antibodies not bound are removed by washing. Bound antibodies of the IgG class are detected by incubation of the substrate with fluorescein-labelled, anti-human IgG conjugate. Reactions are observed under a fluorescence microscope equipped with appropriate filters.

The presence of ANA, ASMA, AMA, AGPA and LKM antibodies is demonstrated by an apple green fluorescence of specific histologic structures in the tissue. The titre (the reciprocal of the highest dilution giving a positive reaction) is then determined by testing serial dilutions<sup>21</sup>.

## PRODUCT INFORMATION

### Storage and preparation

Store all reagents at 2 - 8°C. Reagents are ready for use after equilibration to room temperature.

### Materials provided

<b>SORB</b>   <b>SLD</b>   <b>8</b>	<b>Code: 2152</b>	8 well Mouse Kidney/Stomach Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>10</b>	<b>Code: 2152-1</b>	10 well Mouse Kidney/Stomach Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>8</b>	<b>Code: 2152-3</b>	8 well Mouse Kidney/Stomach/Liver Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>10</b>	<b>Code: 2152-10</b>	10 well Mouse Kidney/Stomach/Liver Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>8</b>	<b>Code: 2153</b>	8 well Mouse Liver, Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>6</b>	<b>Code: 2167</b>	6 well Mouse Kidney Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>8</b>	<b>Code: 2167-8</b>	8 well Mouse Kidney Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>6</b>	<b>Code: 2169</b>	6 well Mouse Stomach Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>10</b>	<b>Code: 2190</b>	10 well HEp-2 / Mouse Kidney/Stomach Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>8</b>	<b>Code: 2190LKM</b>	8 well HEp-2 / Mouse Kidney/Stomach/Liver Substrate Slide.
<b>SORB</b>   <b>SLD</b>   <b>10</b>	<b>Code: 2191</b>	10 well HEp-2 / Mouse Kidney Substrate Slide.

## PERFORMANCE CHARACTERISTICS

The ImmuGlo™ Autoantibody Test System (Mouse Kidney/Stomach Sections) was compared with another commercially available fluorescent antibody test using mouse kidney/stomach as a substrate. The comparison included: 20 samples of ANA positive sera, 19 samples of AMA positive sera, 19 samples of ASMA positive sera, 20 samples of AGPA positive sera and 38 serum samples from normal subjects. Sera were tested starting at a 1:10 dilution with the procedure recommended by the manufacturer. These yielded comparable results as summarized in Tables 8 and 9.

**Table 8. Findings in Positive Sera**

	n	Negatives			Positives	
		<10	10-20	Titre 40-80	160-320	640-2560
<b>ANA Positive Sera</b>						
IMMCO	20	0	0	7	6	7
Other	20	0	0	7	8	5
<b>AMA Positive Sera</b>						
IMMCO	19	4	1	3	1	10
Other	19	4	1	3	4	7
<b>ASMA Positive Sera</b>						
IMMCO	19	3	4	8	4	0
Other	19	2	5	6	5	1
<b>AGPA Positive Sera</b>						
IMMCO	20	0	1	6	4	9
Other	20	0	2	6	7	5

**Table 9. Findings in Normal Controls**

	n	Negatives		Positives	
		<10	10-20	Titre 40-80	>160
<b>ANA Positive Sera</b>					
IMMCO	38	30	3	5	0
Other	38	36	0	2	0
<b>AMA Positive Sera</b>					
IMMCO	38	38	0	0	0
Other	38	38	0	0	0
<b>ASMA Positive Sera</b>					
IMMCO	38	35	2	1	0
Other	38	29	8	1	5
<b>AGPA Positive Sera</b>					
IMMCO	38	37	0	1	0
Other	38	37	0	1	0

**Table 5. Incidence of Anti-Gastric Parietal Cell Antibodies (AGPA) as Detected by Indirect Immunofluorescence on Mouse Stomach Substrate**

Clinical Condition	% Positive
Pernicious Anaemia (PA)	85-95
Chronic Atrophic Gastritis without PA	30-60
Gastric Ulcer	25-30
Autoimmune Endocrinopathies	25-33
Sjögren's Syndrome	30
First Degree Relatives of PA Patients	30
Normal Controls	
< 20 years old	2
20-60 years old	6-8
> 60 years old	16

**Table 6. Incidence of Thyroglobulin and Microsomal Antibodies**

Clinical Condition	% Positive
Hashimoto's disease	80-90
Viral thyroiditis	Transient
Radiation thyroiditis	Transient
Reidel's thyroiditis	Transient
Non-toxic nodular goitre	20
Non-goiterous multifocal thyroiditis	85
Thyroid cancer	20-50
Grave's disease	50-70
Normal Controls	
< 20 years old	2
20-60 years old	6-8
> 60 years old	16

Anti-LKM antibodies are found in a subgroup of patients with idiopathic autoimmune chronic hepatitis<sup>18,20</sup>. The majority of patients with LKM antibodies had clinical liver disease. Some LKM antibody patients may have sub-clinical hepatitis or hepatocellular carcinoma. LKM antibodies identify a subgroup of HBsAg-negative chronic active hepatitis in which other autoantibody markers are absent. In addition these patients exhibit low levels of serum IgA. See Table 7.

**Table 7. Characteristics of LKM Antibody Positive Patients**

Sex (F/M)	4:1	Condition	% Incidence
Age	Mean 34 years		
HBsAg		Negative	
		Anti-nuclear	12%
		Anti-smooth muscle	18%
		Anti-mitochondrial	0%
		Anti-thyroglobulin	12%
		Anti-microsomal	35%
		IgG	Normal
		IgM	Normal
		IgA	Low
		Immunoglobulin levels (g/dL)	

### Material required and available from CLS

<b>CONTROL + ANA</b>	1 x 0.5mL ANA Positive Control, homogeneous pattern. <a href="#">Code 2201</a>
<b>CONTROL + ANA</b>	1 x 0.5mL ANA Low Titre Control, homogeneous pattern. <a href="#">Code 2201-1</a>
<b>CONTROL + ANA</b>	1 x 0.5mL ANA Positive Control, speckled pattern. <a href="#">Code 2202</a>
<b>CONTROL + ANA</b>	1 x 0.5mL ANA Positive Control, nucleolar pattern. <a href="#">Code 2204</a>
<b>CONTROL + ANA</b>	1 x 0.5mL ANA Positive Control, peripheral pattern. <a href="#">Code 2205</a>
<b>CONTROL + AMA</b>	1 x 0.5mL AMA Positive Control, anti-mitochondrial. <a href="#">Code 2210</a>
<b>CONTROL + LKM</b>	1 x 0.5mL LKM Positive Control, liver kidney microsomal. <a href="#">Code 2242</a>
<b>CONTROL -</b>	1 x 0.5mL Negative Control. <a href="#">Code 2200</a>
<b>IgG-CONJ FITC</b>	1 x 5mL Anti-human IgG FITC Conjugate. <a href="#">Code 2100X</a> or
<b>IgG-CONJ FITC EB</b>	1 x 5mL Anti-human IgG FITC Conjugate containing Evan's Blue. <a href="#">Code 2100</a>
<b>BUF</b>	1 x 60mL Buffered Diluent. <a href="#">Code 2302</a>
<b>BUF WASH</b>	1 vial Phosphate Buffered Saline (PBS). Dissolve each vial to 1 litre. <a href="#">Code 2301</a>
<b>MOUNTING MEDIUM</b>	1 x 5.0mL Mounting Medium. Do not freeze. <a href="#">Code 2505</a>
<b>EVANS</b>	1 x 1.0mL Evan's Blue Counterstain. Not required if using 2100. <a href="#">Code 2510</a>

### Material required but not provided

Fluorescence microscope  
 Micropipette or Pasteur pipette  
 Serological pipettes  
 Staining dish (e.g. Coplin jar)  
 Small test tubes (e.g. 13 x 75mm) and test tube rack  
 Distilled or deionised water  
 1 litre container

Wash bottle  
Paper towels  
Incubation chamber  
Coverslip

## WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.  
For Professional Use only.

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. All human serum specimens and human derived products should be treated as potentially hazardous, regardless of their origin. Follow good laboratory practices in storing, dispensing and disposing of these materials<sup>19</sup>.

**WARNING** - Sodium azide (NaN<sub>3</sub>) is present in reagents at <0.1%. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide build-up. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control centre.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange components with those from other sources other than the same catalogue number from IMMCO/CLS. Do not use beyond expiration date given on the labels.

## SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly haemolysed, lipaemic or microbial contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2 - 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

## PROCEDURE

### Test Method

#### A. Screening

1. Dilute each patient serum 1:10 with the Buffered Diluent (20µL serum + 200µL Diluent). **Do not dilute Positive or Negative Controls.** Save the undiluted sera to determine antibody titres if screening tests are found to be positive.
2. Allow pouches containing substrate slides to equilibrate to room temperature for 10 - 15 minutes. Carefully remove the slides without touching the substrate.
3. Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent drying.

**Table 2: Incidence of Antinuclear Antibodies (ANA) Detected by Indirect Immunofluorescence on Mouse Kidney Substrate**

Clinical Condition	No. of Sera	% Positive
SLE	21	95
Scleroderma	17	82
Rheumatoid Arthritis	20	5
Normal Controls	96	0

**Table 3: Incidence of Anti-Mitochondrial Antibodies (AMA) Detected by IFA on Mouse Kidney Substrate**

Clinical Condition	% Positive
Primary Biliary Cirrhosis	100
Autoimmune Chronic Active Hepatitis	8
HBSAg and Chronic Active Hepatitis	0
Extrahepatic Jaundice and Other Liver Diseases	0
Systemic Lupus Erythematosus (SLE)	3
Rheumatoid Arthritis	0
Normal Controls	0

Adapted from Meyer zum Büschenfelde KH, et al.<sup>21</sup>; Walker JG, et al.<sup>22</sup> and Paronetto F and Popper H<sup>23</sup>.

**Table 4: Incidence of Anti-Smooth Muscle Antibodies (ASMA) as Detected by IFA on Mouse Kidney Substrate**

Clinical Condition	% Positive
Chronic Active Hepatitis (Type A)	50 - 87
Primary Biliary Cirrhosis	25
Acute Viral Hepatitis	87
Infectious Mononucleosis	87
Burkitt's Lymphoma	73
Nasopharyngeal Carcinoma	23
Hodgkin's Disease	23
Myeloproliferative Disorder	5
Warts	4
Normal Controls	3 - 18

Adapted from Anderson P, et al.<sup>13</sup>

AMA may be observed on both the distal and proximal tubules of the kidney with the distal tubules staining more brightly. Even though the cytoplasm of the gastric parietal cells also stains, AMA should be quantitated on the kidney.

Staining of the stomach muscularis and kidney glomeruli may also be observed with ASMA, but only ASMA seen on the blood vessel walls of the kidney should be reported.

### LIMITATION OF THE PROCEDURE

In some cases, sera positive for ANA may either be very weak or negative at the initial screening dilution (prozone phenomenon). In such doubtful cases the sera should be screened at higher dilutions and, if positive, antibody titres determined.

In some cases the presence of two or more antibodies in a serum which are reactive with the same substrate may cause interference in their detection by immunofluorescence. This interference may cause either failure to detect ANA or suppression of its titre if the interfering antibody has a higher titre than ANA. All ANA reactions should be reported.

The goat anti-human IgG FITC conjugate supplied in this kit is primarily heavy chain specific but has some light chain activity. It reacts primarily with IgG class autoantibodies, but may, to a lesser degree, react with light chains of other classes such as IgM.

A positive ANA should not be considered indicative of SLE by itself. They also occur in patients with other connective tissue diseases and certain drugs such as procainamide and hydralazine may induce a positive ANA<sup>1</sup>. Moreover, sera of patients with malignancies and infectious diseases may also have a positive ANA.

The clinician should consider the results of all positive indirect immunofluorescence tests along with the results of other laboratory tests and the clinical condition of the patient when making a diagnosis.

### EXPECTED VALUES

As seen in Tables 2, 3, 4, 5 and 6, tests for nuclear antibodies are used to screen for SLE and certain other immunologic disturbances. AMA occurs in over 90% of cases of primary biliary cirrhosis and 3-11% of cases of chronic hepatitis. ASMA occur in the majority of cases of chronic active hepatitis and AGPA are commonly associated with pernicious anaemia and chronic atrophic gastritis.

4. Invert dropper vial and gently squeeze to apply 1-2 drops (each drop is approximately 50µL) of Negative Control to well #1. Similarly apply 2 drops of Positive Control to well #2. Avoid overfilling the wells.
5. Using a micropipette or Pasteur Pipette, apply 1-2 drops of patient's diluted serum to the other wells. Avoid overfilling the wells.
6. Place the lid on the incubation chamber and incubate slides for 30 minutes at room temperature (18 – 25°C).
7. Remove slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately 10mL PBS using a pipette, or rinse slide in beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash for 10 minutes. Repeat process with all remaining slides.
8. Remove slide(s) from Coplin jar. Blot the edge of the slide on a paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the Conjugate dropper vial and gently squeeze to apply 1-2 drops to each well.
9. Replace the lid on the incubation chamber. Incubate for 30 minutes at room temperature (18 – 25°C).
10. Remove a slide from incubator. Hold the slide at the tab end and dip the slide in a beaker containing PBS to remove excess conjugate. Place slide(s) in a staining dish filled with PBS for 10 minutes. If desired, 2 - 3 drops of Evans blue counterstain may be added to the final wash. Repeat for the remaining slides.  
NOTE: Improper washing may lead to increased background fluorescence.
11. Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **To prevent slide from drying, proceed immediately with next step while slide is still wet.**
12. Mount the coverslip by applying 3 drops of Mounting Medium evenly on the coverslip and place coverslip over slide. Avoid applying undue pressure and prevent lateral movement of the coverslip.
13. Repeat steps 11 and 12 for each slide.
14. Examine for specific fluorescence under a fluorescence microscope at a magnification of 200x or greater.

Slides may be read as soon as prepared. However, because of the presence of antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed for up to 48 hours. Slides should be stored in the dark at 2 - 8°C.

## B. Endpoint Determination (titration)

A serum positive in the screening test may be further tested following steps 5 through 12 to determine the titre. Each test run should include the Positive and Negative Controls. Make serial two-fold dilutions starting at 1:10. The reciprocal of the highest dilution producing a positive reaction is the titre.

### Preparation of Serial Dilutions

Number six tubes 1 through 6. Add 1.0mL of Buffered Diluent to tube 1 and 0.2mL to tubes 2 through 6. Pipette 0.1mL of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2mL from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2mL from one tube to the next after mixing to yield the dilutions depicted in the following table:

Tubes	1	2	3	4	5	6
Serum	0.1mL +					
Buffered Diluent	1.0mL	0.2mL	0.2mL	0.2mL	0.2mL	0.2mL
Transfer		⇌ 0.2mL	⇌ 0.2mL	⇌ 0.2mL	⇌ 0.2mL	⇌ 0.2mL
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320 etc

Contact CLS for help with protocols for use with automated IFA instrumentation.

## QUALITY CONTROL

Both a Positive and Negative Control should be included with each test run. The Negative Control should show no specific fluorescence of the nuclei, smooth muscle, tubules of the kidney or gastric parietal cells. The AMA Positive Control should have 2+ or greater staining intensity of the tubules of the kidney. The ANA Positive Control should have 2+ or greater staining intensity of the nuclei of the kidney and liver with a predominantly homogeneous pattern.

If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

- Turbidity. Discard and use another control
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, bulb beyond useful life expectancy, etc.
- Allowing the slide to dry during the procedure.

## INTERPRETATION OF RESULTS

The results of the tests for ANA, AMA, ASMA, AGPA and anti-LKM antibodies should be reported as negative (< 10) on kidney and stomach sections,

negative (<20) on liver sections, positive (>320) , or alternatively, positive with titre.

Read only fields which contain specific staining of the nuclei for ANA, the kidney tubules for AMA, the blood vessel walls for ASMA and gastric parietal cells only for AGPA, liver parenchymal cells and kidney proximal tubules for LKM antibodies. All other reactions should be reported as negative for ANA, AMA, ASMA and/or AGPA.

ANA can be detected on all substrates but should be quantified on the kidney or liver. The nuclear staining patterns observable with the kidney or liver substrate provided include homogeneous, peripheral (rim), speckled and nucleolar. They may be one or a combination of several staining patterns. The latter are due to reactions to several different nuclear antigens.

**Homogeneous:** The entire nucleus fluoresces evenly with a diffuse staining pattern.

**Peripheral (rim):** The nuclear membrane stains most intensely with decreasing staining intensity of the nucleoplasm towards the centre of the nucleus.

**Speckled:** Discrete coarse to fine round speckles fluoresce throughout the nucleus.

**Nucleolar:** The nucleoli stain as multiple solid bodies within the nucleus.

The specificity of some of the antibodies giving the above staining patterns may be further identified by tests for antibodies to nDNA and to various extractable nuclear antigens. These may be of diagnostic significance as listed in table 1.

**Table 1. Diagnostic Significance of Antinuclear Antibodies**

IF Staining Pattern	Nature of Antigen	Associated Disease
Homogeneous	Deoxyribonucleoprotein	SLE with renal involvement
Peripheral	DNA	SLE
Speckled	RNP	SLE or MCTD*
	Sm	SLE
	SS-A/SS-B	SLE or Sjögren's
	Scl-70	Scleroderma
Nucleolar	4S-6S RNA probably U3 RNA	Scleroderma

\*Mixed Connective Tissue Disease

LKM antibodies exhibit a characteristic granular cytoplasmic staining of the liver parenchymal cells and proximal tubules of the kidney. The reactions on stomach are usually negative.