

6. Manns M, Gerken G, Kyriatsoulis A and Meyer zum Büschenfelde KH. Significant autoimmune markers of autoimmune liver disorders: Current status. J Clin Lab Anal 1: 362-370, 1987.
7. Mackay IR. Autoimmunity and the liver. Clin Aspects Immunity 2: 8- 17, 1988.
8. McMillan SA, Alderdice JM, McKee CM et al. Diversity of autoantibodies in patients with anti-mitochondrial antibody and their diagnostic value. J Clin Path 4: 232-236, 1987.
9. Gershwin ME, Coppel RL and Mackay IR. Primary biliary cirrhosis and mitochondrial autoantigens - insights from molecular biology. Hepatology 8: 147-151, 1988.
10. Berg PA and Klein R. Mitochondrial antigens and autoantibodies from anti-M1 to anti-M9. Klin Wochenschr 64: 897-909, 1986.
11. Popper H and Paronetto F. Clinical, histologic and immunopathologic features of primary biliary cirrhosis. Springer Semin Immunopathol 3: 339-354, 1980.
12. Berg PA and Bacon H. Serology of primary biliary cirrhosis. Springer Semin Immunopathol 3: 355-373, 1980.
13. Anderson P, Small JV and Sobieszek A. Studies on the specificity of smooth muscle antibodies. Clin Exp Immunol 22: 22-29, 1975.
14. Kurki P, Miettinen A, Linder E, Pikkarainen P, Vuoristo M and Salaspuro MP. Different types of smooth muscle antibodies in chronic active hepatitis and primary biliary cirrhosis: Their diagnostic and prognostic significance. Gut 21: 878-884, 1980.
15. Fisher JB and Taylor KB. The significance of gastric antibodies. Brit J Haematol 20: 1-7, 1971.
16. Chisholm M. Immunology of gastritis. Clin Gastroenterol 5: 419-428, 1976.
17. Bigazzi PE, Burek CL and Rose NR. Antibodies to tissue-specific endocrine, gastrointestinal and neurological antigens. In "Manual of Clinical Laboratory Immunology". Rose NR, Friedman H and Fahey JL, Eds, American Society for Microbiology, Washington DC, 762-770, 1986.
18. Beutner EH, Kumar V, Krasny SA and Chorzelski TP. Defined immunofluorescence in immunodermatology. In "Immunopathology of the Skin", Beutner EH, Chorzelski TP and Kumar V, Eds, John Wiley and Sons, New York, 3rd Ed, 3-40, 1987.
19. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control, National Institutes of Health, [HHS Pub. No. (CDC) 93-8395] 1993.
20. Nisengard RJ. Antinuclear antibodies: Significance of titers. In "Immunopathology of the Skin", Beutner EH, Chorzelski TP and Bean S, Eds, John Wiley and Sons, New York, 2nd Ed, 387-398, 1979.
21. Meyer zum Büschenfeld KH, Manns M and Trautman F. Autoimmunity in chronic liver diseases - relationship to SLE? In "Recent Advances in Systemic Lupus Erythematosus". Lambert PH, Perrin L, and Izui S, Academic Press, New York, 259-269, 1984.
22. Walker JG, Doniach D, Roitt IM and Sherlock S. Serologic tests in the diagnosis of primary biliary cirrhosis. Lancet 1: 827, 1965.
23. Paronetto F and Popper H. Hetero-iso- and autoimmune phenomena in the liver. In "Textbook of Immunopathology", Miescher PA and Müller- Eberhard HJ, Eds, Grune and Stratton, New York, 2nd Ed, 789-817, 1976.

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@immcodiagnostics.com

or your local product distributor



ImmuGlo™

AUTOANTIBODY TEST SYSTEM

For *in vitro* Diagnostic Use

PRODUCT INSERT

<i>Code: 1100</i>	Mouse Liver Substrate Kit	48 Det
<i>Code: 1107</i>	COMVI Mouse Kidney/Stomach Substrate	48 Det
<i>Code: 1136</i>	COMVI-III Mouse Kidney/Stomach/Liver Substrate	48 Det

INTENDED USE

Indirect immunofluorescence antibody tests for the detection and quantitation of anti-nuclear antibodies (ANA), *Cat. No. 1100, 1107, 1136*, anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), and anti-gastric parietal cell antibodies (AGPA) in human serum, *Cat. No. 1107, 1136*.

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 scleroderma¹⁻⁵. 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 cirrhosis⁶⁻⁸.

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 extra-hepatic biliary obstruction and in other liver diseases. The universal presence of anti-mitochondrial antibodies in primary biliary cirrhosis and their virtual absence in extra-hepatic jaundice makes their detection of considerable value in the differential diagnosis⁶⁻¹².

Anti-smooth muscle antibodies (ASMA) in high titer (≥ 160) occur in the majority of cases of chronic active hepatitis and in intermediate titers (40-80) in acute viral hepatitis. Occasionally they may occur in cases of primary biliary cirrhosis where they are found in intermediate titers. The significance of titers of 20-40 is doubtful since these titers may occur in normal individuals^{13,14}.

Anti-gastric parietal cell antibodies (AGPA) are commonly associated with pernicious anemia 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 anemia¹⁵⁻¹⁷.

PRINCIPLES OF PROCEDURE

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

The presence of ANA, ASMA, AMA and AGPA is demonstrated by an apple green fluorescence of specific histologic structures in the tissue. The titers (the reciprocal of the highest dilution giving a positive reaction) are then determined by testing serial dilutions of the serum¹⁸.

REAGENTS

Storage and Preparation

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

Precautions

For *in vitro* Diagnostic Use. 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 materials¹⁹.

WARNING - Sodium azide (NaN₃) 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 buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO Diagnostics. Do not use beyond expiration date.

Table B. Findings in Normal Controls

	n	P o s i t i v e T i t e r			
		Negative < 10	10-20	40-80	>160
ANA Positive					
IMMCO	38	30	3	5	0
Other	38	36	0	2	0
AMA Positive					
IMMCO	38	38	0	0	0
Other	38	38	0	0	0
ASMA Positive					
IMMCO	38	35	2	1	0
Other	38	29	8	1	0
AGPA Positive					
IMMCO	38	37	0	1	0
Other	38	37	0	1	0

Sera obtained from 96 normal subjects, 21 patients with SLE, 17 patients with scleroderma and 20 patients with rheumatoid arthritis were tested on the antinuclear antibody (ANA) test (mouse liver sections) kit and other commercially available ANA kits. Sera were tested according to the procedure and screening dilution recommended by the manufacturers. These yielded comparable results as indicated in Table C.

Table C. Comparison of Kits Using Tissue Sections or HEp-2 Cells for the Detection of ANA

Clinical Condition	n	P o s i t i v e		
		% IMMCO Mouse Liver	Other Mouse Kidney	Other HEp-2
SLE	21	95	95	86
Scleroderma	17	82	88	77
RA	20	25	25	5
Normal Controls	96	0	0	0

REFERENCES

1. Tan EM. Autoantibodies to nuclear antigens (ANA): Their immunobiology and medicine. *Adv Immunol* 33: 167-240, 1982.
2. Kumar V, Beutner EH and Chorzelski TP. Autoimmunity of the skin. In "Concepts in Immunopathology", Vol 1, Cruse JM and Lewis RE Jr, Eds, Karger, Basel, 318-353, 1985.
3. Reimer G, Cornell RC and Tan EM. The biochemical nature of nuclear antigens reactive with antinuclear antibodies. In "Immunopathology of the Skin", Beutner EH, Chorzelski TP and Kumar V, Eds, John Wiley and Sons, New York, 3rd Ed, 519-531, 1987.
4. Beutner EH, Kumar V, Krasny SA and Chorzelski TP. Standardization of antinuclear antibody and other immunofluorescent tests used in immunopathologic studies of the skin. In "Immunopathology of the Skin", Beutner EH, Chorzelski TP and Kumar V, Eds, John Wiley and Sons, New York, 3rd Ed, 41-64, 1987.
5. Tan EM, Chan EKL, Sullivan KF and Rubin RL. Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. *Clin Immunol Immunopathol* 47: 121-141, 1988.

Table 4. Incidence of Anti-Gastric Parietal Cell Antibodies (AGPA) as Detected by Indirect Immunofluorescence on Mouse Stomach Substrate

Clinical Condition	% Positive
Pernicious Anemia (PA)	85-95
Chronic Atrophic Gastritis without PA	30-60
Gastric Ulcer	25-30
Autoimmune Endocrinopathies	25-33
Sjogren'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

PERFORMANCE CHARACTERISTICS

The 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 Table A and B

Table A. Findings in Positive Sera

	Negative		P o s i t i v e			
	n	< 10	10-20	40-80	160-320	640-2560
ANA Positive						
IMMCO	20	0	0	7	6	7
Other	20	0	0	7	8	5
AMA Positive						
IMMCO	19	4	1	3	1	10
Other	19	4	1	3	4	7
ASMA Positive						
IMMCO	19	3	4	8	4	0
Other	19	2	5	6	5	1
AGPA Positive						
IMMCO	20	0	1	6	4	9
Other	20	0	2	6	7	5

Materials Provided

6 x	8-well Substrate Slides,	<i>Code: 1100, 1107</i>
8 x	6-well Substrate Slides,	<i>Code: 1136</i>
1 x 0.5 ml	ANA Positive Control*.	
1 x 0.5 ml	AMA Positive Control*.	<i>Code: 1107, 1136</i>
1 x 0.5 ml	Negative Control*.	
1 x 5.0 ml	Goat anti-human IgG FITC Conjugate, heavy and light chain specific. Ready to use. Protect from light* .	
1 x 60 ml	Buffered Diluent. Ready to use*.	
2 vials	Phosphate Buffered Saline. Dissolve each vial to 1 liter.	
1 x 5.0 ml	Mounting Medium. Do not freeze* .	
1 x 1.0 ml	Counterstain	
12 x	Coverslips	

*Contains <0.1% NaN₃

Materials 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 75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

Recommended Optional Materials

- Speckled ANA Positive Control, *Code: 2202*
- Nucleolar ANA Positive Control, *Code: 2204*
- Peripheral ANA Positive Control, *Code: 2205*
- ANA Control Pattern II, *Code: 1603*, 1 each homogeneous, speckled, peripheral and nucleolar pattern controls
- ASMA Positive Control, *Code: 2211*
- AGPA Positive Control, *Code: 2212*

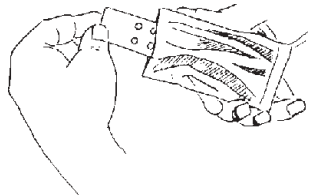
SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially 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.

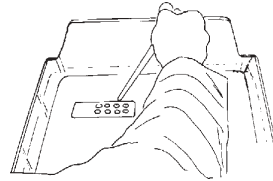
PROCEDURE

Test Method

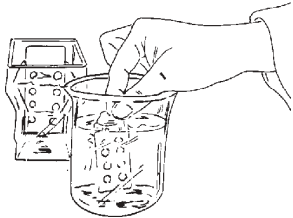
The indirect immunofluorescence staining procedure is illustrated in the following figures:



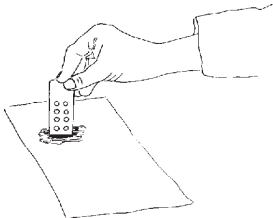
1. Let pouch equilibrate to room temperature, then remove slide(s) from pouch.



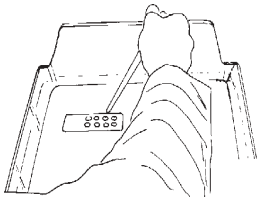
2. Place slide(s) into moisture chamber and add samples and controls. Cover and incubate 30 minutes.



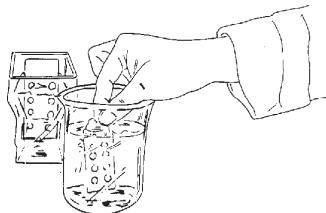
3. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



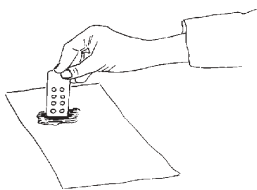
4. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



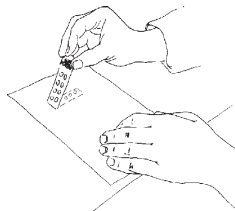
5. Apply Conjugate to each well. Cover and incubate 30 minutes.



6. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



7. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



8. Mount cover slip and read under fluorescent microscope.

EXPECTED VALUES

As seen in Tables 1, 2, 3 and 4 below, tests for nuclear antibodies are used to screen for SLE and certain other immunologic disturbances. AMA occur 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 anemia and chronic atrophic gastritis.

Table 1: 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 2: Incidence of Anti-Mitochondrial Antibodies (AMA) Detected by Indirect Immunofluorescence 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.²¹; Walker JG, et al.²² and Paronetto F and Popper H²³.

Table 3: Incidence of Anti-Smooth Muscle Antibodies (ASMA) as Detected by Indirect Immunofluorescence 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.¹³

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 (*Please consult IMMCO Catalog for Product Codes*). These may be of diagnostic significance as listed below:

Diagnostic Significance of Antinuclear Antibodies

Immunofluorescence 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
	ScI-70	Scleroderma
Nucleolar	4S-6S RNA	Scleroderma
	probably U3 RNA	

*Mixed Connective Tissue Disease

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.

LIMITATIONS OF THE PROCEDURE

In some cases, positive sera 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 titers determined.

In some ANA positive sera two or more antibodies may be present in varying titers as indicated by different staining patterns. All nuclear antibody patterns and their titers should be reported.

A positive ANA should not be considered diagnostic 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 ANA¹. Moreover, sera of patients with malignancies and infectious diseases may also have positive ANA²⁰. 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.

A. Screening:

Step 1. Dilute each patient serum:

- a. **1:10** (*Code: 1107 and 1136*) with the buffered diluent provided (20 µl serum + 180 µl diluent).
- b. **1:20** (*Code: 1100*) with the buffered diluent provided (10 µl serum + 190 µl diluent).

Do not dilute Positive or Negative Controls. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.

Step 2 Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides without touching the substrate.

Step 3 Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent slides from drying.

Step 4 Invert dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) of the **Negative Control** to well #1. Similarly apply **1 drop** of **ANA Positive Control** to well #2 and, if applicable **1 drop** of **AMA Positive Control** to well #3 for *Code: 1107 and 1136*. Avoid overfilling the wells.

Step 5 Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling the wells.

Step 6 Place the lid on the incubation chamber and incubate slides **30 minutes** at room temperature.

Step 7 Remove a slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately **10 ml** of PBS using a pipette, or rinse slide in a beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash **10 minutes**. Repeat process with all remaining slides.

Step 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 drop** (approximately 50 µl) to each well. Repeat process with all remaining slides.

Step 9 Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.

Step 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**. Repeat process with all remaining slides. If desired, 2-3 drops of Evans blue counterstain may be added to the final wash. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 11 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **While slide is still wet mount the coverslip.** Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any movement of the coverslip. Repeat process with all remaining slides

Step 12 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 an antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2°- 8°C.

B: End Point Determination (Titration)

A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each test run should include the appropriate Positive and Negative Controls. Make serial two-fold dilutions starting at **1:10** for *Cat. No. 1107,1107-1 and 1136*, or **1:20** for *Cat. No. 1100*. Using one slide, a serum may be tested at dilutions ranging from 1:10 to 1:320. If positive at a 1:320 dilution, the titer is reported as greater or equal to 320. Alternatively, additional slides may be used to obtain endpoints for those sera still positive at a 1:320 dilution. The reciprocal of the highest dilution producing a positive reaction is the titer.

Preparation of Serial Dilutions

Number six tubes 1 through 6. Add 0.9 ml of buffered diluent to tube 1 and 0.2 ml to tubes 2 through 6. Pipette 0.1ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield the dilutions depicted below:

Tubes	1	2	3	4	5	6
Serum	0.1 ml					
	+					
Buffered Diluent	0.9 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Transfer		0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320 etc.

Quality Control

Both a positive and negative control serum should be included with each test run. The negative control should show no 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:

- Contamination as a result of improper storage or handling. If signs of contamination such as turbidity are seen, discard and use another control.
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life, etc.
- Allowing the slide to dry during the procedure.

RESULTS

The results of the tests for ANA, AMA, ASMA, and AGPA should be reported as negative (< 10) on kidney and stomach substrates (*Code: 1107 and 1136*), negative (<20) on liver sections (*Code: 1100*), or alternatively, positive with titer.

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. All other reactions should be reported as negative for ANA, AMA ASMA and/or AGPA.

The nuclear staining patterns observable include homogeneous, peripheral (rim), speckled and nucleolar and are described below. Sometimes there may be 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 center of the nucleus.

Speckled: Discrete coarse to fine round speckles in the the nucleus.

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