AUTO I.D.® SSA/Ro and SSB/La AUTOANTIBODY TEST SYSTEM

For in vitro Diagnostic Use
For Professional Use

INTENDED USE: This is an Ouchterlony immunodiffusion test system for the detection of autoantibodies to SSA/Ro, SSB/La, and other autoantigens in human serum. The results from this test system can be used as an aid in the diagnosis of Systemic Lupus Erythematosus, Sjögren's Syndrome, or other rheumatic diseases.

Summary and Explanation of the Test

Cellular proteins that are soluble in saline are called Extractable Nuclear Antigens or ENA. Antibodies to ENA have been associated with several autoimmune syndromes, and appear to be of diagnostic and/or prognostic significance in systemic sclerosis (1, 2), mixed connective tissue disease (3-5), Sjögren's syndrome (6, 7), polymyositis (8), dermatomyositis (9), systemic lupus erythematosus (5), and rheumatoid arthritis (10). Some of the more common antibody specificities include: Smith (Sm); Ribonucleoprotein (RNP or U1-RNP); SSA/Ro; SSB/La; Jo-1; Scl-70; and proliferating cell nuclear antigen (PCNA) (11). The antinuclear antibody (ANA) test has been used as a screen for these antibodies, but the ANA does not indicate the specificity of the antibody, and antibodies to some ENA do not show a positive ANA test (12, 13). Thus, secondary confirmatory testing for antibodies to ENA is highly recommended (14).

SSA and SSB were originally described as nuclear RNA-protein antigens in patients with Sjögren's syndrome (6, 7). Ro and La were described as cytoplasmic RNA-protein antigens in patients with SLE (15, 16). It is now widely accepted that SSA and Ro are analogous, SSB and La are analogous, and these antigens are found in both the nucleus and cytoplasm. Antibodies to SSA/Ro alone or SSA/Ro and SSB/La are found in up to 70-90% of patients with subacute cutaneous lupus (11, 17), and in 85% of patients with Sjögren's syndrome who develop vasculitis (18). Antibodies to SSA/Ro alone occur in patients who have a homozygous deficiency of the C2 complement fraction (11, 19), in primary biliary cirrhosis patients who develop Sjögren's syndrome (20), and in up to two thirds of patients with "ANA negative SLE" (21).

Principle of the Test

A number of assays are available for the detection of specific antibodies to nuclear antigens. The most commonly used methods include Ouchterlony immunodiffusion, passive hemagglutination, counterimmunoelectrophoresis, and ELISA (14). The Ouchterlony immunodiffusion method (ID) is currently the most widely used assay due to its convenience and relative ease of performance and interpretation.

The Immuno Concepts SSA/Ro and SSB/La AUTO I.D.® Autoantibody Test System for the detection of antibodies to SSA/Ro and SSB/La nuclear antigens uses the Ouchterlony immunodiffusion technique. The test employs Immuno Concepts' exclusive nuclear antigen preparation that contains a variety of nuclear antigens that react with antibodies found in systemic rheumatic disease patients. Nuclear antigen is placed in a central well of an agarose plate with control sera and patient serum samples placed in the surrounding six sample wells. After incubation at room temperature, a line of precipitation forms in the agarose gel where nuclear antigen diffuses and meets homologous antibody. Sera are tested for antigen/antibody specificity by viewing precipitation lines of identity or partial identity between patient samples and control sera. Sera that do not produce precipitation lines are considered negative. Antibodies with different specificities may produce lines of non-identity when compared to control sera used in the assay.

System Components - materials provided

Use: All control sera come ready to use with no diluting, aliquoting, or reconstitution required. The nuclear antigen comes lyophilized and must be reconstituted with distilled or deionized water prior to use.

Storage: All components can be stored in the refrigerator at 2-10°C. Reconstituted nuclear antigen should be used within 5 days or stored frozen in aliquots at –20°C or less.

Stability: All control sera are stable for at least 12 months from date of manufacture. Agarose plates are stable for 24 months from date of manufacture. Lyophilized nuclear antigen is stable at least 12 months from date of manufacture. After reconstitution, nuclear antigen is stable for 5 days at 2-10°C, or 90 days frozen at –20°C or less. For best results store reconstituted antigen in 30 µl aliquots at –20°C or less.

REACTIVE REAGENTS

AUTO I.D.® Nuclear Antigen ANTIGEN: Cat. No. 6050 (0.2 ml). Lyophilized mammalian extracted nuclear antigen containing Smith (Sm), U1-Ribonucleoprotein (RNP), SSA/Ro, SSB/La, and other antigens that commonly react with antibodies from patients with systemic rheumatic disease. Each vial must be reconstituted with 200 µl of distilled or deionized water prior to use.

> **Preparation:** Remove the metal cap from the nuclear antigen vial. Carefully lift the rubber stopper to vent the vial. Remove the stopper and add 200 µl of distilled or deionized water to the vial. Replace the rubber stopper and gently swirl to dissolve contents. Allow reconstituted antigen to sit at least 30 minutes prior to use to insure antigen is completely dissolved. Reconstituted antigen may appear turbid or cloudy. Swirl immediately before use.

SSA/Ro/SSB/La Positive Control CONTROL| + : Cat. No. 7002 (0.5 ml). Ready-to-use vial containing human antibodies reactive with SSA/Ro and SSB/La nuclear antigens. This serum demonstrates strong precipitation lines of identity to CDC reference sera for these antigens.

SSA/Ro Positive Control CONTROL| + : Cat. No. 7001 (0.5 ml). Ready-to-use vial containing human antibodies reactive with SSA/Ro nuclear antigen. This serum demonstrates a single strong precipitation line of identity to CDC reference sera for this antigen.

NON-REACTIVE COMPONENTS

AUTO I.D.® Plates PLATE: Cat. No. 7010. Seven-well agarose plates that include pre numbered wells to ensure easy identification of patients.

> **Preparation:** Allow plate to reach room temperature (18-25°C) prior to opening foil pouch. Carefully remove plate from foil pouch. Condensation present on the inside lid of the plate can be removed with bibulous paper or lint-free paper toweling. Avoid touching the agarose.

Additional Materials Required - but not provided

Volumetric pipettes to deliver 20 µl, 30 µl, 100 µl, and 200 µl volumes
Test tubes
Deionized or distilled water
Immunodiffusion light box and magnifier
Disposable gloves

Optional Components Available

In the event positive undefined precipitin line results are obtained, additional control sera are available to aid in determination of antibody specificity. Repeat the test with appropriate ready-to-use control sera placed in the wells adjacent to the patient sample and interpret following the guidelines found in the “General Interpretation” section.

SSB/La Positive Control Serum CONTROL| + : Cat. No. 7003 (0.5 ml). Ready-to-use vial containing human antibodies reactive with SSB/La nuclear antigen.

RNP Positive Control Serum CONTROL| + : Cat. No. 6001 (0.5 ml). Ready-to-use vial containing human antibodies reactive with U1-Ribonucleoprotein (RNP) nuclear antigen.

Sm/RNP Positive Control Serum CONTROL| + : Cat. No. 6002 (0.5 ml). Ready-to-use vial containing human antibodies reactive with Smith (Sm) and U1-Ribonucleoprotein (RNP) nuclear antigens.

Jo-1 Positive Control Serum CONTROL| + **:** Cat. No. 6004 (0.5 ml). Ready-to-use vial containing human antibodies reactive with Jo-1 antigen.

Scl-70 Positive Control Serum CONTROL| + **:** Cat. No. 6005 (0.5 ml). Ready-to-use vial containing human antibodies reactive with Scl-70 nuclear antigen.

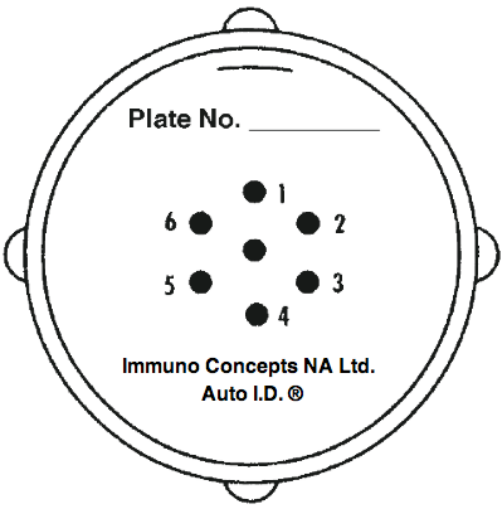
PCNA Positive Control Serum CONTROL| + **:** Cat. No. 6006 (0.5 ml). Ready-to-use vial containing human antibodies reactive with proliferating cell nuclear antigen (PCNA).

Precautions

1. All human source materials used for this product have been tested and found to be negative (not repeatedly reactive) for antibodies to Human Immunodeficiency Virus-1 (HIV-1), Human Immunodeficiency Virus-2 (HIV-2), hepatitis C virus (HCV) and for hepatitis B surface antigen (HBsAg) by FDA approved methods. However, no test method can offer complete assurance that HIV-1, HIV-2, hepatitis C, hepatitis B, or other infectious agents are absent. Therefore, all kit materials should be handled in the same manner as potentially infectious material.
2. Sodium azide (0.09%) is used as a preservative. When disposing of reagents, flush with ample volumes of tap water to prevent potential residues in plumbing.
3. Do not freeze AUTO I.D.® plates. To assure consistent results, always warm plates to room temperature prior to use.
4. Avoid repeated freeze/thawing of reconstituted nuclear antigen.
5. Always allow freshly reconstituted nuclear antigen to sit at least 30 minutes at room temperature prior to use to make sure antigen is completely dissolved.
6. Substitution of components from other Immuno Concepts AUTO I.D.® Autoantibody Test Systems is acceptable. Substitution of components from other manufacturers may yield inconsistent results.
7. Abrupt changes in air temperature may cause artifact precipitin lines to form. For best results, incubate plates in a controlled temperature environment protected from air currents. Do not incubate at 37°C.
8. Some sera may demonstrate false negative results due to a prozone (antibody excess) phenomenon. If the prozone phenomenon is a concern, repeat the test using dilutions of patient serum in PBS.
9. Some patient sera containing phospholipids may form wide bands of precipitation surrounding the entire patient well. This should not be interpreted as a positive reaction.
10. This test system is for *in vitro* diagnostic use.

Methods of Testing

AUTO I.D.® testing can be set up in one-step and two-step protocols to achieve minimum turn-around time or maximum economy, respectively. The following are recommended as general guidelines to aid in setting up a protocol optimal to each laboratory's specific requirements.



Low Volume Screening and/or Confirmation Testing (Method 1)
Well 1 - Patient 1
Well 2 - Monospecific antibody control (SSA/Ro)
Well 3 - Patient 2
Well 4 - Mixed antibody control (SSA/Ro/SSB/La)
Well 5 - Patient 3
Well 6 - Mixed antibody control (SSA/Ro/SSB/La)
Center Well - Nuclear antigen

High Volume Screening and/or Titering (Method 2)
Well 1 - Patient 1
Well 2 - Patient 2
Well 3 - Patient 3
Well 4 - Patient 4
Well 5 - Patient 5
Well 6 - Mixed antibody control (SSA/Ro/SSB/La)
Center Well - Nuclear antigen

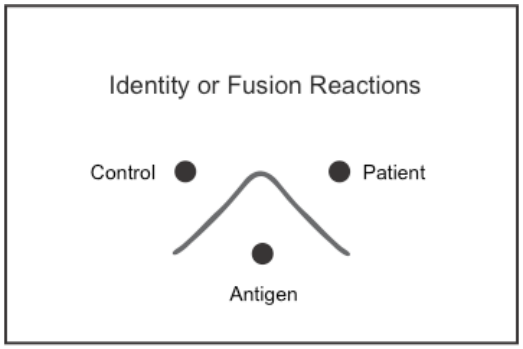
Those patient sera demonstrating precipitin lines by Method 2 after 18-24 hours should be further tested for specificity by following Method 1.

Specimen Collection

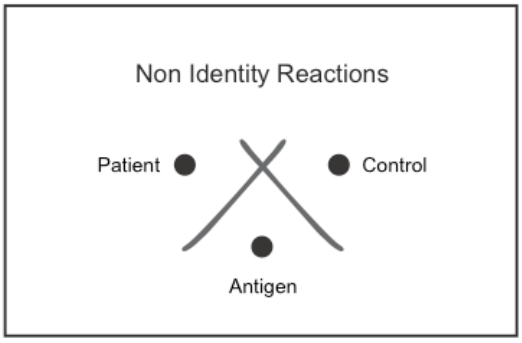
Serum should be collected by aseptic technique. Serum should be separated from the clot as soon as possible to prevent hemolysis. Sera exhibiting a high degree of hemolysis, lipemia, or microbial growth should not be used. Sera may be stored at 2-10°C up to 48 hours prior to use. If testing is further delayed, sera should be stored frozen at –20°C or less.

Results – General Interpretation

Proper interpretation of patient results depends on clear resolution of the precipitin line between patient serum and nuclear antigen wells. Determination of the patient antibody specificity depends on proper interpretation of precipitin lines between patient serum and adjacent control wells. The following definitions should serve as a basic guideline to interpretation of reactions between patient sera and control sera.

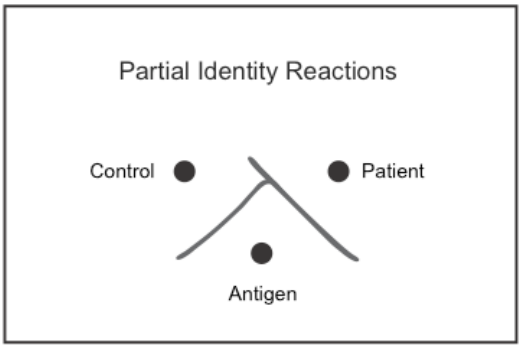


Precipitin lines that form a continuous line between patient and control sera indicate antibodies in each serum are reacting with identical nuclear antigens. Patient samples demonstrating precipitin lines of identity are reported positive with antibody specificity identical to the control.



Precipitin lines that cross between patient and control sera indicate antibodies in each serum are reacting with different nuclear antigens.

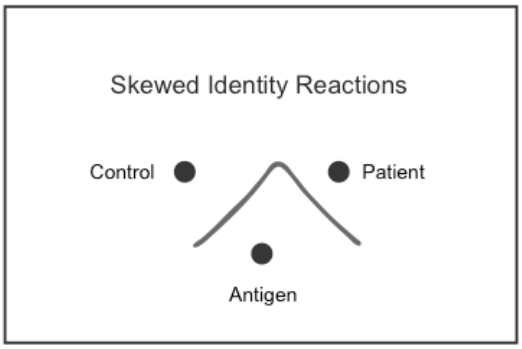
Samples demonstrating precipitin lines of non-identity are reported positive with “undefined precipitin line” (UPL) reactivity. Further testing is recommended with other controls to determine antibody specificity (See “Optional Components Available”).



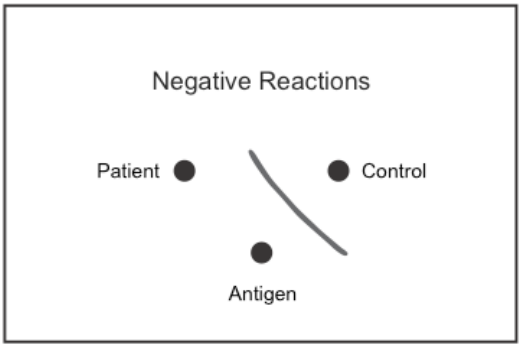
Precipitin lines that form a “spur” between patient and control wells indicate antibodies in the patient and control sera are reacting with an identical antigen, but the patient serum also contains an antibody that reacts with a different antigen that does not react with the control serum.

CAUTION: Partial identity reactions are the most difficult reactions to interpret. Often the control precipitin line will curve into the patient precipitin line at the point of contact. View the precipitin lines carefully to make sure the patient’s precipitin line does not cross the control’s precipitin line.

Precipitin lines that form a skewed continuous line between patient and control wells indicate each serum is reacting with identical nuclear antigens, but the patient serum contains more or less antibody than the control serum.



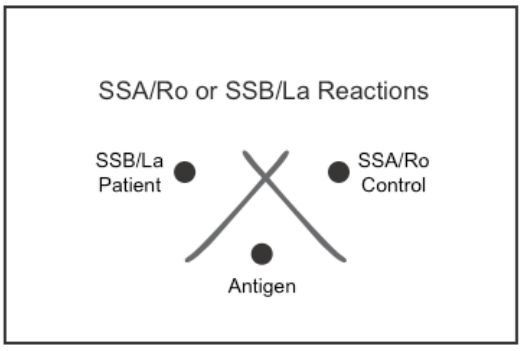
Patient samples demonstrating skewed lines of identity are reported positive with specificity identical to the control.

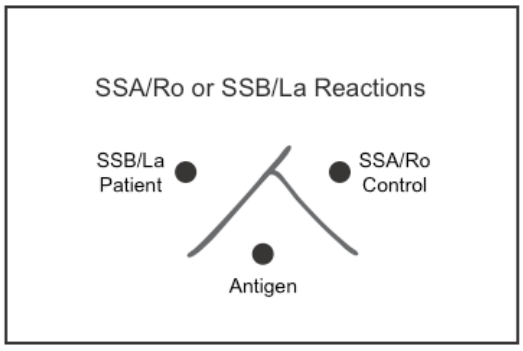


A precipitin line forms with the control serum only. Patient samples that do not form precipitin lines are reported negative.

Technical Interpretation

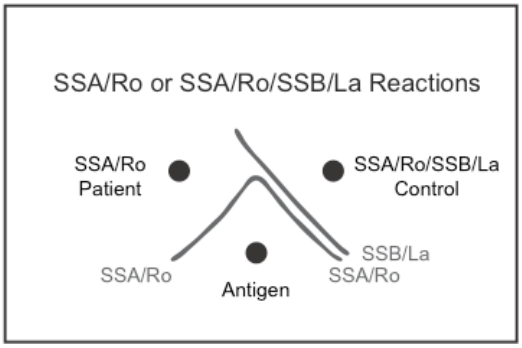
SSA/Ro or SSB/La
Antibodies to SSA/Ro and SSB/La will generally demonstrate precipitin lines that cross, indicating non-identity. SSA/Ro precipitin lines are commonly seen as narrow and sharp while SSB/La precipitin lines may appear wider and less defined.





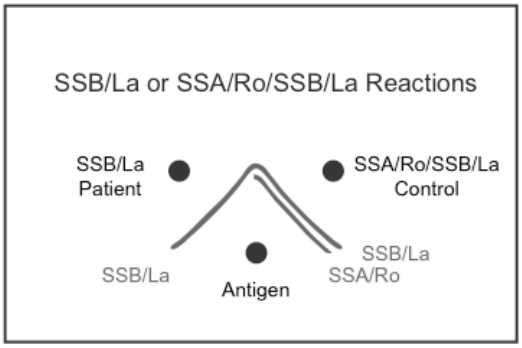
SSA/Ro or SSA/Ro/SSB/La
Sera monospecific for SSA/Ro antibodies are generally differentiated from sera that contain antibodies to both SSA/Ro and SSB/La by assessing the precipitin characteristics, and the reactivity with control sera. Sera with antibodies to SSA/Ro alone will usually form one narrow, sharp precipitin line with identity to the SSA/Ro control.

Sera with antibodies to both SSA/Ro and SSB/La will demonstrate one narrow precipitin with identity to the SSA/Ro control and one wide precipitin line that shows identity to the SSB/La control. In some sera that are positive for both SSA/Ro and SSB/La antibodies, the two precipitin lines may appear to demonstrate partial fusion.



SSB/La or SSA/Ro/SSB/La

Differentiation of sera that are monospecific for SSB/La antibodies from sera that contain SSA/Ro/SSB/La antibodies can be difficult because the SSB/La precipitin line is generally wider and less defined than the SSA/Ro precipitin line. To confirm that a serum contains both SSA/Ro and SSB/La antibodies, both precipitin lines must be clearly visible. Generally, a wide precipitin line demonstrating identity to the SSB/La control and a narrow precipitin line demonstrating identity to the SSA/Ro control are observed. The SSA/Ro precipitin line may appear to partially fuse with the SSB/La precipitin. Serial dilution of sera may improve differentiation of the two precipitin lines.



Titering

Serial two-fold dilutions of patient sera can be used to provide a semi-quantitative determination of the amount of specific antibody present in positive sera. Titering may also aid in interpretation of reactions occurring close to the nuclear antigen well on initial screening due to antibody excess. Report titer as the reciprocal of the last dilution that shows clear precipitin lines of identity to control sera.

Limitations of the Test

1. Although a positive result may be suggestive of a systemic rheumatic disease, it should not be considered diagnostic, but rather viewed as part of the overall clinical profile of a patient.
2. The Immuno Concepts AUTO I.D.® test systems are optimized to detect the majority of patients with autoantibodies to Sm, RNP, SSA/Ro, SSB/La, Scl-70, Jo-1 and PCNA antigens. Occasional samples with very high or very low antibody levels may give false negative results in any Ouchterlony immunodiffusion test system. Dilution of samples in PBS or concentration of antibody by double or triple filling the patient wells may enhance detection of antibody in these samples.
3. The Immuno Concepts AUTO I.D.® nuclear antigen includes a blend of mammalian autoantigens. Thus, patient sera may demonstrate precipitin lines with antigens that do not show identity reactions with the control sera included in this test system. Such sera should be retested with control sera to other antigen specificities (see “Optional Components Available”).

Expected Values

Immunospecificity of Autoantibodies to Nuclear Antigens (Data from Reference 14)	
Antibodies to:	**Disease Association:**
Sm	SLE: 25-40%; marker antibody
Nuclear RNP (U1-RNP)	MCTD: 95-100%; lower frequency in SLE, discoid lupus, PSS
SSA/Ro	Sjögren's Syndrome: 60-70%; SLE: 30-40%; neonatal lupus syndrome: 100%
SSB/La	Sjögren's Syndrome: 50-60%; SLE: 10-20%
PCNA	SLE: 10%; marker antibody
Scl-70	PSS: 15-20%; marker antibody
Jo-1	Polymyositis: 31%; marker antibody
PM-Scl (PM-1)	Polymyositis/Scleroderma overlap: 64%;

Abbreviations: SLE = systemic lupus erythematosus, MCTD = mixed connective tissue disease, PSS = progressive systemic sclerosis, Sm = Smith antigen, PCNA = proliferating cell nuclear antigen.

Performance Characteristics

Detection: The Immuno Concepts SSA/Ro/SSB/La AUTO I.D.® Autoantibody Test System was tested with a total of 61 positive and negative patient sera obtained from qualified reference laboratories (22). There was a 96.7% agreement with all sera tested. Eight sera were positive for SSB/La antibodies, one serum was positive for SSA/Ro and SSB/La antibodies, and three sera were positive for SSA/Ro antibodies. Fifteen sera demonstrated "undefined precipitin lines" (UPLs). On further testing with Immuno Concepts' Sm/RNP AUTO I.D.® Autoantibody Test System, twelve of these sera were positive for RNP antibodies, two sera were positive for Sm/RNP antibodies, and one serum was positive for Sm antibodies. Thirty-two sera were negative for any detectable autoantibodies to nuclear antigens. The two discrepant sera were originally reported positive for SSA/Ro and SSA/Ro/SSB/La antibodies respectively. These sera were further tested on two other tests in commercial distribution and gave unclear precipitin lines when compared to SSA/Ro, SSB/La, Sm, and U1-RNP prototype controls (clear lines of identity or non-identity could not be detected).

Precision: Six sera positive for SSA/Ro/SSB/La antibodies were tested in duplicate on three occasions. In all cases, all test results demonstrated identical antibody specificities: five sera were uniformly positive for SSA/Ro and SSB/La antibodies, and one serum was uniformly positive for SSB/La antibodies.

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In the event of damage to the protective packaging, please contact Immuno Concepts prior to use.

 Manufacturer

EC REP Authorized Representative in the European Community

 Temperature Limitation

 Contains Sufficient for <n> tests

 Consult Instructions for Use

IVD In Vitro Diagnostic Medical Device

EC REP MDSS GmbH
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D-30175 Hannover, Germany

CE

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Technical Support USA: 1.800.251.5115 Outside USA: 1.916.363.2649
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Cat 7000-I, 4.11.02.003.097-En Rev 3.0

AUTO-I.D.® Test Procedure

1. **RECONSTITUTE NUCLEAR ANTIGEN**
   Reconstitute the vial of nuclear antigen by adding 200 µl of distilled or deionized water. Allow reconstituted vial to sit at least 30 minutes at room temperature (18-25°C) prior to use to ensure antigen is completely dissolved. Swirl gently before use.
   NOTE: Reconstituted antigen may appear turbid or cloudy. For best results store reconstituted antigen in 30 µl aliquots at –20°C or less. Allow antigen to reach room temperature prior to use.

2. **PREPARE AUTO I.D.® PLATE(S)**
   Allow plate to reach room temperature (18-25°C) prior to opening foil pouch. Carefully remove plate from foil pouch. Condensation present on the inside lid of the plate can be removed with bibulous paper or lint-free paper toweling. Avoid touching the agarose.

3. **PREPARE AUTO I.D.® WORKSHEET**
   Record the plate number, control specificity by well number, and patient identification by well number for each sample to be tested. Also record the lot number of the AUTO I.D.® Autoantibody Test System being used onto the AUTO I.D.® worksheet.

4. **DILUTE PATIENT SAMPLES (OPTIONAL)**
   Dilutions of patient serum samples may be desired for titering or if a prozone (antibody excess) phenomenon is observed. Prepare dilutions of patient samples with phosphate buffered saline (PBS). Dilute patient sample 1:2 by adding 100 µl of undiluted patient sample to 100 µl of PBS. To continue titering make two-fold serial dilutions of the serum sample (e.g., 1:2,1:4,1:8 ... 1:64) using PBS.

5. **FILL WELLS**
   Place 20 µl of nuclear antigen in center well of AUTO I.D.® plate. Place 20 µl of patient sample or control serum in numbered wells following one of the recommended formats outlined under “Methods of Testing.” Replace lid.

6. **DOUBLE FILLING PATIENT SAMPLES (OPTIONAL)**
   Occasional samples with very low antibody levels may give very weak or false negative results in any Ouchterlony immunodiffusion test system. Concentration of antibody by double or triple filling the patient wells may enhance detection in these samples. Concentration of the samples can be achieved by refilling the patient well with an additional 20 µl of serum after approximately 30 minutes.

7. **INCUBATE PLATES**
   Carefully place filled plates in a small box to protect them from air currents and incubate at room temperature (18-25°C) for 18-24 hours. Do not incubate at 37°C.
   CAUTION: Air currents and abrupt changes in air temperature may cause artifact precipitin lines to form. For best results, incubate plates in a controlled temperature environment.

8. **READ PLATES**
   View plates on a light box with a magnifier after 18-24 hours. Refer to the “Interpretation” section for recommended guidelines for reading precipitin lines.
   NOTE: For most sera, results should be visible within 18 hours. With some low-titered sera, more distinct precipitin lines may be seen at 24 and 48 hours.

FOR TECHNICAL ASSISTANCE:
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Email: technicalsupport@immunoconcepts.com