

For *In Vitro* Diagnostic Use

Intended Use

NOVA Gel™ Scl-70 is a double diffusion test system for the titering and semi-quantitative determination of autoantibodies to Scl-70, an extractable nuclear antigen (ENA), in human serum. The presence of antibodies to Scl-70 can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of scleroderma.

Summary and Explanation of the test

The term "Anti-nuclear antibodies" (ANA) describes a variety of autoantibodies that react with constituents of cell nuclei including DNA, RNA and several proteins and ribonucleoproteins.¹ These autoantibodies occur with high frequency in patients with connective tissue or rheumatic diseases, especially SLE. Virtually all SLE patients are ANA positive. This diagnostic sensitivity has led to the incorporation of ANA testing into the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus by an Arthritis and Rheumatism Association subcommittee.² While ANA testing is an excellent screening test for SLE (a negative result virtually rules out active SLE³) it is by no means a specific test.

The current trend in diagnosing and managing connective tissue disease is to combine a sensitive ANA screening test with more specific follow up tests such as dsDNA and/or ENA. The ENA tests are especially important as they provide both diagnostic and prognostic information.

Antibodies to Scl-70 are a specific marker for scleroderma,^{2,4} much as antibodies to Sm and native DNA are specific marker autoantibodies for SLE. The Scl-70 antibody is found in 20-60% of scleroderma patients.^{2,4,5,6} The wide range in the reported frequency of Scl-70 antibody may be due to differences in assay sensitivity.⁷ When scleroderma patients are divided into those with the milder CREST Syndrome variant and the more severe diffuse or progressive systemic scleroderma, Scl-70 antibodies are found to be more prevalent in diffuse scleroderma patients (>70%) compared to the milder disease group.^{6,8}

Principles of the Procedure

The double diffusion test, described by Ouchterlony,⁹ allows an antigen solution to passively diffuse through a gel support matrix towards a patient sample and/or antibody containing control. Specially shaped wells punched into the gel matrix serve as reservoirs from which the antigen and antibody solutions diffuse towards each other. At the point of antigen-antibody equivalence, a visible precipitin line forms in the gel. The immunologic status of a patient can then be determined by observing the patterns of adjacent precipitin lines formed in the gel by a control sample of known specificity and an unknown patient serum.

The double diffusion technique can be used with a purified Scl-70 preparation, a known Scl-70 autoantibody control sample and a patient serum suspected to contain Scl-70 autoantibodies, to show immunologic identity or non-identity. Confirmation of Scl-70 autoantibody activity in the patient's serum can be made if its precipitin line characteristically merges with the line formed by a known control sample.

In certain instances, a semi-quantitative determination of the amount of Scl-70 autoantibody in a patient's serum is desired. In these cases the NOVA Gel “T” plate can be utilized to assay a serially diluted patient sample. The reciprocal of the highest dilution which forms a precipitin line can then be reported as the titer of the Scl-70 autoantibody for the patient. Correct double diffusion testing procedure calls for the assay of multiple antigen-antibody concentration ratios in order to prevent antigen or antibody excess. Incorrect or misleading precipitin line formation can occur from antigen or antibody excess. To prevent this, dilutions of the patient serum should be assayed against the same antigen preparation. This is concurrently accomplished while titering the patient's sample on the NOVA Gel “T” plate.

Reagents

1. Nova Gel “T” screening gel plates with 7 wells each, containing stabilizers and 0.09% sodium azide
2. 0.4mL Scl-70 antigen (from calf thymus), lyophilized in buffer containing stabilizers and 0.09% sodium azide
3. 1.4mL Scl-70 Control, human serum containing autoantibodies to Scl-70 in buffer containing 0.09% sodium azide.

Warnings

1. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HbsAg, and HCV by FDA cleared methods. No test method, however, can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Scl-70 Control should be handled in the same manner as potentially infectious material.¹⁰
2. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. It is recommended that **sterile distilled or deionized** water be used to rehydrate the antigen.
4. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the prevention of reagents or patient samples overflowing the wells and the quality of the water used to rehydrate the antigen. Careful attention to consistency is required to obtain accurate and reproducible results.
5. Strict adherence to the protocol is recommended.

Storage Conditions

Store all the kit reagents at 2-8°C. **Do not freeze.** Reagents are stable until the expiration date when stored and handled as directed. Once rehydrated, the antigen is stable for 2 weeks at 2-8°C. If desired, 100µL aliquots of antigen can be prepared and stored at $\leq -70^{\circ}\text{C}$. Antigen stored in this manner is stable for at least 1 year.

Specimen Collection

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure

Materials provided

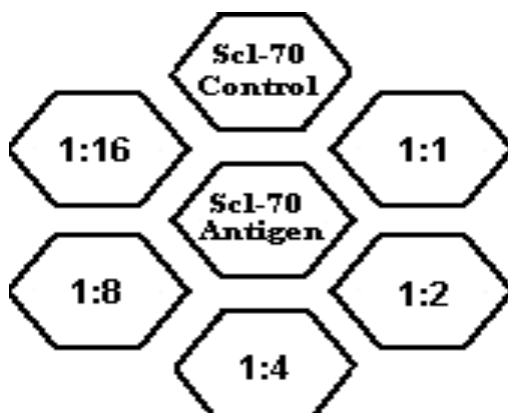
- 8 Prepunched NOVA Gel titrating "T" plates
- 2 0.4mL Scl-70 antigen, lyophilized
- 1 1.4mL Scl-70 Control

Additional Materials Required But Not Provided

- Micropipets to deliver 15-100µL volume
- Disposable micropipet tips
- 0.85% Saline
- Distilled or deionized water (preferably sterile)
- Light source or gel viewing box

Method

1. **Rehydrate the Scl-70 Antigen**
Carefully open an antigen vial and add 0.4mL of distilled or deionized water to the lyophilized antigen. Sterile distilled or deionized water is highly recommended for optimal product performance. Allow the rehydrated antigen to stand for 5 minutes, then swirl to mix thoroughly before use.
2. **Filling the Gel Plate**



Prepare a serial twofold dilution of the patient sample in 0.85% saline. Load the gel plate wells with antigen (approximately 80µL), control (approximately 80µL), and patient sample dilutions (approximately 80µL) as illustrated. Exact fill amounts are not critical; however, **DO NOT OVERFLOW THE WELLS**. The control may be filled directly from plastic squeeze bottles or micropipets. If using squeeze bottles, place the dispenser tip into the well and gently squeeze until the control level nears the top of the well. Cover the plate immediately after filling.

3. **Incubation**
Allow the covered plate(s) to incubate at room temperature on a stable, flat surface for 24 hours. Examine the plate(s) and note any precipitin lines that form between the patient sample and the antigen. Examine the relationship of this line to the control's precipitin line. Reexamine the plate(s) again at 48 hours to check for any newly developed lines prior to final interpretation of the results.

Quality Control

Scl-70 Control should be run on every plate to insure that all reagents and procedures have performed properly. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at $\leq -70^{\circ}\text{C}$. In order for the test results to be considered valid, a precipitin line must be visible between

the Scl-70 and the Scl-70 antigen wells.

Interpretation of Results

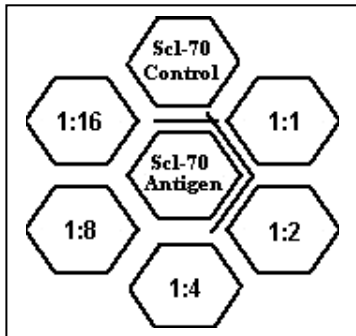
For optimal observation of the precipitation lines, the following procedure is recommended:

1. Remove the plate lid.
2. Hold the plate in a position which minimizes the gel surface reflection.
3. Illuminate the gel plate from the side (i.e. parallel to the surface of the gel). Note: Be sure the light source is shielded from direct view.
4. Precipitin lines are best seen when contrasted against a dark background and viewed from an angle.
5. A magnifier may aid in the observation of faint lines or for distinguishing non-identity samples.

Negative Reaction. A sample is considered negative if no visible precipitin line forms between the patient wells and the antigen well after 48 hours.

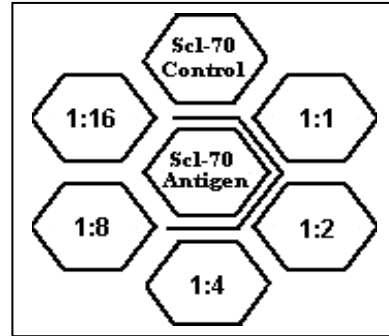
Specificity Interpretation. In double diffusion, specificity is determined by noting the relationship of the sample precipitin lines with the known control lines. The following possibilities can occur with the NOVA Gel™ Scl-70 test:

Fig. 1: Non-Identity



The patient precipitin line crosses or shows **non-identity** with the Scl-70 control. This indicates that the patient reacts with an ENA antigen other than Scl-70.

Fig. 2: Scl-70 Identity



The patient precipitin line merges or shows **identity** with the Scl-70 control line. In this case the patient has autoantibodies to Scl-70.

Limitations of the Procedure

1. Although unlikely with this test because of the serial dilution of the sample, prozoning is a possibility. Prozone occurs when an overwhelming amount of patient autoantibody is present relative to the antigen (antibody excess), causing the precipitin line to form at the antigen well face. In these cases retesting of possible positive samples at higher dilutions in saline is recommended to prevent a false negative test interpretation.
2. If non-sterile or contaminated water is used to rehydrate the antigen, the antigen may suffer immediate or progressive degradation. In this case a very light precipitin line or no line may result.
3. Results of this assay should be used in conjunction with clinical findings and other serological tests.
4. The assay performance characteristics have not been established for matrices other than serum.

Expected Values

NOVA Gel™ Scl-70 double diffusion test was used to evaluate a variety of connective tissue disease patients as well as 200 random blood donors. The results appear below:

| Patient Group | Number | Number Positive NOVA Gel™ ENA Test Scl-70 Positive |
|----------------------|--------|---|
| SLE | 105 | 0 |
| Drug Induced Lupus | 24 | 0 |
| Rheumatoid Arthritis | 40 | 0 |
| Scleroderma | 24 | 15 |
| Dermatomyositis | 14 | 0 |
| Sjogren's Syndrome | 14 | 0 |
| Normals | 200 | 0 |

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