Immuno Concepts is proud to announce that when present the characteristic SS-A/Ro pattern detected by the HEP-2000® substrate is now considered confirmatory for the presence of antibodies to the SS-A/Ro antigen.
Autoantibodies to SS-A/Ro can be present in a variety of autoimmune diseases including Sjögren’s syndrome, systemic lupus erythematosus (SLE), subacute cutaneous lupus, and neonatal lupus syndrome. While the etiology of the antibodies and exact pathology caused by these antibodies remains elusive, the evidence linking SS-A/Ro autoantibodies to disease is undeniable, making detection of these autoantibodies a critical step in the diagnosis.

Screening for autoantibodies to SS-A/Ro has traditionally been done using substrates such as rodent tissue or cultured cell lines. These substrates have been shown to be less than reliable for detection of autoantibodies to SS-A/Ro alone. Efforts to improve sensitivity for SS-A/Ro autoantibodies have previously relied on adjustments to the fixation process, which often resulted in decreased morphological quality of metaphase mitotic cells.

Transfection is a multi-step process that leads to a new gene (or genes) being inserted into a host cell. In the case of human SS-A/Ro, the gene of interest is the one responsible for expression of the native 60kD Ro peptide. This gene was inserted into HEp-2 cells to create the HEp-2000® cells. Some of the cells receive multiple copies of the gene causing these cells to produce large quantities of the 60 kD Ro/SS-A autoantigen. We refer to these cells as hyperexpressing cells. This transfection does not affect the expression of the other nuclear and cytoplasmic antigens.

This guide is designed to give the laboratorian and the physician a complete understanding of the HEp-2000® substrate.

**Commentary on SS-A/Ro Detection**

Autoantibodies to SS-A/Ro can be present in a variety of autoimmune diseases including Sjögren’s syndrome, systemic lupus erythematosus (SLE), subacute cutaneous lupus, and neonatal lupus syndrome. While the etiology of the antibodies and exact pathology caused by these antibodies remains elusive, the evidence linking SS-A/Ro autoantibodies to disease is undeniable, making detection of these autoantibodies a critical step in the diagnosis. Screening for autoantibodies to SS-A/Ro has traditionally been done using substrates such as rodent tissue or cultured cell lines. These substrates have been shown to be less than reliable for detection of autoantibodies to SS-A/Ro alone. Efforts to improve sensitivity for SS-A/Ro autoantibodies have previously relied on adjustments to the fixation process, which often resulted in decreased morphological quality of metaphase mitotic cells.

Transfection is a multi-step process that leads to a new gene (or genes) being inserted into a host cell. In the case of human SS-A/Ro, the gene of interest is the one responsible for expression of the native 60kD Ro peptide. This gene was inserted into HEp-2 cells to create the HEp-2000® cells. Some of the cells receive multiple copies of the gene causing these cells to produce large quantities of the 60 kD Ro/SS-A autoantigen. We refer to these cells as hyperexpressing cells. This transfection does not affect the expression of the other nuclear and cytoplasmic antigens.

At this 1:40 dilution only the 10-15% of cells which hyperexpress the SS-A/Ro autoantigen demonstrate staining of the nucleus and nucleoli (a). Some may show staining in the cytoplasm. The non-hyperexpressing cells are negative (b). There is no staining in the chromosome area of the metaphase mitotic cells (c). This is now reported as ANA positive, SS-A/Ro pattern. The presence of autoantibodies to SS-A/Ro is confirmed when this distinct ANA pattern is seen. Follow-up ENA testing is suggested to rule out the presence of antibodies to other ENA’s. Because there is little correlation between ANA titer and disease severity, titering of the SS-A/Ro pattern is not necessary. When seen alone, the SS-A/Ro pattern will not mask over other ANA patterns.

We suggest reporting this sample as follows:
- ANA positive
- SS-A/Ro pattern present, titering is not necessary
- ENA testing is suggested to rule out the presence of autoantibodies to other ENAs.
Speckled and SS-A/Ro Mixed Pattern Seen on HEp-2000®

At the 1:40 dilution the 10-15% of cells which hyperexpress the SS-A/Ro autoantigen demonstrate stronger staining of the nucleus and nucleoli (a). Some may show staining in the cytoplasm. The non-hyperexpressing cells demonstrate speckled staining (b). There is no staining in the chromosome area of the metaphase mitotic cells (c). This is a mixed pattern. ANA positive. The two patterns present are speckled and SS-A/Ro.

At the 1:80 dilution both the speckled and the SS-A/Ro pattern are present.

At the 1:160 dilution the speckled pattern is fading but still visible. The SS-A/Ro pattern is still clearly visible.

At the 1:320 dilution the speckled pattern has disappeared leaving only the SS-A/Ro pattern.

Because of the speckled pattern we suggest titering this sample and reporting the titer endpoint of the speckled just as you would any mixed ANA pattern. The titer endpoint is the highest dilution where a clearly discernible pattern is visible. The speckled pattern endpoint is at the 1:160 dilution. The SS-A/Ro pattern remains visible through the 1:320 dilution. Because there is little correlation between ANA titer and disease severity, titering of the SS-A/Ro pattern is not necessary. When seen alone, the SS-A/Ro pattern will not mask over other ANA patterns.

We suggest reporting this sample as follows:

- ANA positive
- Speckled 1:160
- SS-A/Ro pattern present; titering is not necessary
- ENA testing is suggested to rule out the presence of autoantibodies to other ENAs.
**Homogeneous and SS-A/Ro Mixed Pattern Seen on HEp-2000®**

At the **1:40** dilution both a homogeneous and SS-A/Ro pattern are visible. The SS-A/Ro pattern is identified by the hyperexpressing cells (d), while the homogeneous pattern is identified by the smooth staining in the nuclei of non-hyperexpressing cells (e), and the smooth staining in the chromosome area of the metaphase mitotic cells (f). This is a mixed pattern, ANA positive. The two patterns are homogeneous and SS-A/Ro.

At the **1:80** dilution both the homogeneous and the SS-A/Ro pattern are present.

At the **1:160** dilution the homogeneous pattern is fading but still visible. The SS-A/Ro pattern is still clearly visible.

At the **1:320** dilution only the SS-A/Ro pattern is present.

This series of photographs demonstrates how identification of the different patterns can become easier as their relative strengths change during the dilution. The titer endpoint for the homogeneous pattern is **1:160**. The SS-A/Ro pattern remains visible through the **1:320** dilution. Because there is little correlation between ANA titer and disease severity, titering of the SS-A/Ro pattern is not necessary. When seen alone, the SS-A/Ro pattern will not mask over other ANA patterns.

We suggest reporting this sample as follows:

- ANA positive
- Homogeneous 1:160
- SS-A/Ro pattern present; titering is not necessary
- ENA testing is suggested to rule out the presence of autoantibodies to other ENAs.
- dsDNA testing is suggested because of the homogeneous ANA pattern.
The row of photographs at right demonstrates how a strong homogeneous ANA pattern can mask over another ANA pattern. In this case, the homogeneous pattern is masking over an SS-A/Ro pattern.

At the **1:40** dilution a strong homogeneous pattern is seen. Notice that there is smooth staining in the nucleus of the interphase cells (g) and strong staining in the chromosome region of the metaphase mitotic cells (h).

At the **1:80** dilution the strong homogeneous staining continues to dominate. However, notice the nucleolar staining visible in some of the cells (i). This is the SS-A/Ro pattern becoming visible. It was hidden by the stronger homogeneous pattern at the **1:40** dilution.

At the **1:160** dilution the homogeneous pattern is fading and the hyperexpressing cells associated with antibodies to SS-A/Ro are clearly identifiable (j).

At the **1:320** dilution the homogeneous pattern is no longer visible, leaving only the characteristic pattern associated with antibodies to SS-A/Ro. Further titering of this sample continued to show only the SS-A/Ro pattern. Because there is little correlation between ANA titer and disease severity, titering of the SS-A/Ro pattern is not necessary. When seen alone, the SS-A/Ro pattern will not mask over other ANA patterns.

We suggest reporting this sample as follows:
- ANA positive
- Homogeneous 1:160
- SS-A/Ro pattern present; titering is not necessary
- ENA testing is suggested to rule out the presence of autoantibodies to other ENAs.
- dsDNA testing is suggested because of the homogeneous ANA pattern.
REFERENCES

Several articles have been published concerning the usefulness of HEp-2000® as an ANA substrate. Following is a brief summary of the findings published in four of these articles.

The highlights of this article are:
—Details the transfection process for the development of HEp-2000®.
—Describes the distinctive SS-A/Ro pattern.
—Patterns other than SS-A/Ro did not change.
Conclusion:
Transfected substrate is a simple and sensitive method for detection of anti-SS-A/Ro antibodies in patients with systemic rheumatic disease.

The highlights of this article are:
—Describes distinctive SS-A/Ro pattern.
—73 samples with SS-A/Ro antibodies examined.
—69 of 73 identified by distinctive SS-A/Ro pattern.
—Transfection did not significantly alter the pattern(s) typically associated with other autoantibodies.
—Samples with ‘monospecific’ antibodies to 52 kD Ro were also detected by HEp-2000®.
—HEp-2000® also identifies sera that react only with “native Ro particle."
Conclusion:
This new substrate detects SS-A/Ro antibodies that were not identified on standard HEp-2 substrates and by other immunoassays.

The highlights of this article are:
—240 consecutive ANA samples, 100 normal controls and 53 patients diagnosed with primary Sjögren’s syndrome were tested.
—1 of the 100 normals was identified as SS-A/Ro positive by HEp-2000® and confirmed by CIEP.
—14 of the 240 consecutive samples were positive for SS-A/Ro on HEp-2000® and confirmed by CIEP. Three of these 14 samples were negative on standard HEp-2 substrate.
Conclusion:
HEp-2000® is a suitable substrate for ANA testing and has an advantage over regular HEp-2 for the detection of anti-SS-A/Ro autoantibodies. HEp-2000® is a valuable confirmatory test for sera giving equivocal precipitin reactions or ELISA results.

The highlights of this article are:
—160 of 2100 positives (8%) demonstrated the SS-A/Ro pattern and were confirmed SS-A/Ro positive by ELISA.
Conclusion:
HEp-2000® is useful as an ANA screen with a positive predictive value for the SS-A/Ro pattern of 100%. SS-A/Ro antibodies comprise the majority of all ENA antibodies (65%). Over 50% of samples with SS-A/Ro had antibodies to other ENAs.