

QUANTA Lite® SS-B ELISA

708575

For *In Vitro* Diagnostic Use

CLIA Complexity: High

Intended Use

QUANTA Lite® SS-B is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of SS-B antibodies in human serum. The presence of SS-B antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases, such as Sjogren's Syndrome.

Summary and Explanation of the test

Antinuclear antibodies (ANA) are found in a wide variety of connective tissue diseases and as such serve as a sensitive screening assay.¹ While ANA testing is an excellent screening test for SLE (a negative result virtually rules out active SLE)² it is by no means a specific test. Autoantibodies to SS-B antigen are found in 5-15% of SLE patients and 25% of random Sjogren's Syndrome patients.^{3,4} As with SS-A, these antibodies are often times found in patients with clinical features of SLE but are ANA negative.^{5,6} SS-B antibodies are found in approximately 60% of Sjogren's patients with sicca complex.^{7,8}

SS-B antibodies are almost always found to occur simultaneously with SS-A while SS-A can occur alone.⁹ Patients producing both SS-B and SS-A as opposed to SS-A alone generally have a milder disease with lower incidence of nephritis and antibodies to dsDNA.¹⁰

A variety of methods including Ouchterlony double diffusion and passive agglutination have been used to detect antibodies to SS-B. Clinically useful ELISA assays for detecting anti-SS-B antibodies have also been developed. The ELISA technique employed by these assays is objective, semi-quantitative, and can be conveniently used to test large numbers of patients.

Principles of the Procedure

Purified SS-B antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any SS-B antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

Reagents

1. Polystyrene microwell ELISA plate coated with a purified SS-B antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to SS-B, prediluted, 1.2mL
3. SS-B ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to SS-B, prediluted, 1.2mL
4. SS-B ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to SS-B, prediluted, 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
7. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.
2. 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 SS-B ELISA Low Positive, SS-B ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.¹¹
3. 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.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.

5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. 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. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

Storage Conditions

1. 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.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.

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 particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. CLSI (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

- 1 SS-B ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ELISA Negative Control
- 1 1.2mL prediluted SS-B ELISA Low Positive
- 1 1.2mL prediluted SS-B ELISA High Positive
- 1 50mL HRP Sample Diluent
- 1 25mL HRP Wash Concentrate, 40x concentrate
- 1 10mL HRP IgG Conjugate, (goat), anti-human IgG
- 1 10mL TMB Chromogen
- 1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

Additional Materials Required But Not Provided

Micropipets to deliver 5, 100, 200-300 and 500 μ L

Disposable micropipet tips

Test tubes for patient sample dilutions, 4mL volume

Distilled or deionized water

1L container for diluted HRP Wash Concentrate

Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

Method

Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5 μ L of sample to 500 μ L of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the SS-B ELISA Low Positive, SS-B ELISA High Positive and ELISA Negative Control.
4. Determination of the presence or absence of SS-B using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

Assay procedure

1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.**
2. Add 100 μ L of the **prediluted** SS-B ELISA Low Positive, the SS-B ELISA High Positive, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300 μ L of the **diluted** HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100 μ L of the HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100 μ L of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
7. Add 100 μ L of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

Quality Control

1. The SS-B ELISA Low Positive, the SS-B ELISA High Positive and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the SS-B ELISA Low Positive, the SS-B ELISA High Positive and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at $\leq -20^{\circ}\text{C}$.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
 - a. The absorbance of the prediluted SS-B ELISA High Positive must be greater than the absorbance of the prediluted SS-B ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
 - b. The prediluted SS-B ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
 - c. The SS-B ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.

- d. The ELISA Negative Control and SS-B ELISA High Positive are intended to monitor for substantial reagent failure. The SS-B ELISA High Positive will not ensure precision at the assay cutoff.
- e. The user should refer to CLSI (NCCLS) Document C24-A for additional guidance on appropriate QC practices.

Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the SS-B ELISA Low Positive. The result is multiplied by the number of units assigned to the SS-B ELISA Low Positive found on the label.

$$\text{Sample Value (units)} = \frac{\text{Sample OD}}{\text{SS-B ELISA Low Positive OD}} \times \text{SS-B ELISA Low Positive (units)}$$

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient's antibody titer.

Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

The sample can then be classified as negative, weak positive, moderate positive or strong positive according to the table below.

	Units
Negative	<20
Weak Positive	20 – 39
Moderate Positive	40 – 80
Strong Positive	>80

1. A positive result indicates the presence of SS-B antibodies and suggests the possibility of Systemic Lupus Erythematosus (SLE) or related connective tissue diseases.
2. A negative result indicates no SS-B antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite® SS-B ELISA. SS-B values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG levels cannot be correlated to an endpoint titer."

Limitations of the Procedure

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Not all SLE and Sjogren's patients are positive for SS-B. Ouchterlony studies have shown that only 13% (14/105) of SLE patients and 21% (3/14) of Sjogren's patients have SS-B antibody.
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

The ability of the QUANTA Lite® SS-B ELISA to detect SS-B antibodies was evaluated by comparison to a commercially available double diffusion test from INOVA Diagnostics, Inc. Results of the Ouchterlony test were determined as positive if a precipitate line of identity was present and negative if no line or a line of non-identity was observed.

Normal Range

One hundred and one random serum samples were selected and tested by the SS-B ELISA assay. The mean of the sample population was 4.7 units with a standard deviation of 1.1 units. 2.8 to 12.7 units was the range of the population. This trial indicates that the average normal is over 13 standard deviations below the cutoff.

Relative Sensitivity and Specificity

To determine the sensitivity and specificity of the assay, 223 ANA positive patient samples containing antibodies to a wide variety of nuclear antigens were tested by both the Ouchterlony and ELISA methods. Whenever a discrepancy between the Ouchterlony and ELISA results occurred, the samples were further tested by a commercially available ELISA method for specific antibodies against SS-B that might be below the limit of detection of the Ouchterlony technique.

Of the 223 samples tested, 19 were positive and 190 were negative by both methods. The final 14 samples were positive by ELISA but negative in the Ouchterlony assay. Each of these samples was also tested by another commercial ELISA procedure and all 14 showed a positive reaction with SS-B. The results of the assays are summarized in the table below and assumes that the 14 samples positive by both ELISA methods are true positive reactions

		INOVA			
		+	-		
OT	+	33	0	Relative Sensitivity	100%
				Relative Specificity	100%
	-	0	190	Relative Efficiency	100%

Precision and Reproducibility

The precision and reproducibility of the assay was measured by running six replicates each of a strong positive and a weak positive sample in six separate assays. The mean reactivity of the strong positive was 134.6 units while the mean value for the weak positive was 38.7. The standard deviation and coefficient of variation for each sample are summarized below.

	Strong Positive		Weak Positive	
	SD	CV	SD	CV
Overall	3.3	2.4%	0.7	1.9%
Within Run	1.9	1.4%	0.7	1.9%
Between Run	2.9	2.2%	0.6	1.4%

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November 2011
Revision 17

