1.0 INTRODUCTION

The physiologic role of DHEA-S is not well-defined. Serum levels are relatively high in the fetus and neonate, lower in childhood, and increase during puberty [4]. Increased levels of DHEA-S during adolescence may contribute to the development of secondary sexual hair. DHEA-S levels show a progressive decline after the third decade of life [5]. Unlike DHEA, DHEA-S levels do not show significant diurnal variation and little day-to-day variation. DHEA-S levels are not responsive to acute cortisol administration [4], and do not vary significantly during the normal menstrual cycle [2]. This may be due to the slower metabolic clearance of DHEA-S compared to DHEA [6]. Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalinism [3], while elevated levels occur in several conditions: including hyperadrenocorticism and carcinoma [7], 21-hydroxylase and 3α-hydroxysteroid dehydrogenase deficiencies [2,8] and some cases of female hirsutism [2]. Since very little DHEA-S is produced by the gonads [2,3], measurement of DHEA-S may aid in the localization of the androgen source in virilizing conditions. Methods for measurement of DHEA-S include gas-liquid chromatography with flame detection or competitive protein-binding assays, and radioimmunoassay. Although significant inter-method variability occurs with DHEA, androstenedione and androsterone, the relative concentrations of these competing substances in most normal and pathologic samples predicts a minimal effect on assay performance.

The Monobind DHEA-S ELISA Kit uses a specific anti-DHEA-S antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low. The employment of several serum references of known DHEA-S concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with DHEA-S concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for an enzyme immunoassay include antibody, antigen, substrate, enzyme-antigen conjugate and a carrier. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a carrier in solution, a competition results between the antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

\[
\text{Ag} + \text{Ab} + \text{AgAb} \rightarrow \text{AgAb} + \text{A}
\]

\[
A = \text{Biotinylated DHEA-S IgG Antibody (Constant Quantity)}
\]

\[
\text{Ab} = \text{Native Antibody (Variable Quantity)}
\]

\[
\text{AgAb} = \text{Antigen-Enzyme Complex (Variable Quantity)}
\]

\[
\text{AgAb} + \text{A} \rightarrow \text{immobilized complex (A)}
\]

\[
\text{Streptavidin} \rightarrow \text{strep} \rightarrow \text{immobilized complex \& solid surface}
\]

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different concentrations of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. DHEA-S Calibrators – 0 ml/vial

B. DHEA-S Enzyme Reagent – 6 ml/vial

C. DHEA-S Biotin Reagent – 6.0 ml - Icon

D. Streptavidin Coated Plate – 96 wells – Icon

E. Wash Solution Concentrate – 20ml – Icon

F. Substrate A – 7ml/vial – Icon

G. Substrate B – 7ml/vial – Icon

H. Stop Solution – 6ml/vial - Icon

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

5.0 PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human sera should be considered as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control and the American Society for Clinical Laboratory Science. “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8315.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparinized plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a red top vacutainer tube or anti-coagulant (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days, or at -70°C for up to six months. Avoid use of the specimen after freezing and thawing. When assayed in duplicate, 0.020ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assure assays controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test as described above. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh quality control standards should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

2. Working Substrate Solution

Store at room temperature for 1 year

For the contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the contents of the amber vial. Align and label accordingly.

Store at 2 - 8°C.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

**Note:** Assay should be performed by a skilled individual or trained professional!

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.010 ml (10 µL) of the appropriate serum reference, control and patient into a separate well.

3. Add 0.050 ml (50 µL) of the DHEA-S Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Add 0.050 ml (50 µL) of Anti- DHEA-S Biotin Reagent to all wells.

6. Swirl the microplate gently for 20-30 seconds to mix.

7. Cover and incubate for 30 minutes at room temperature.

8. Decant the Substrate B of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 0.50 ml of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to disperse the wash. Decant the wash and repeat two (2) additional times.

10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

11. Incubate at room temperature for fifteen (15) minutes.

12. Add 0.050ml (50µl) of stop solution to each well and gently mix for separate well(s) (see Reagent Preparation Section). Place the plate(s) containing the sample(s) in a separate 37°C water bath and keep the sample(s) in the water bath for thirty (30) minutes of adding the stop solution.

**Note:** Dilute the samples suspected of concentrations higher than 8.0 µg/ml 1:5 and 1:10 with DHEA-S ‘0’ µg/ml calibrator or patient serum pools with a known low value for DHEA-S.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm...
the example, the average absorbance in the patient sample (1.07x) intersects the dose response curve at (1.21 μg/ml) DHEA-S concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**EXAMPLE 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>Well</th>
<th>Abs (A)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>2.562</td>
<td>2.572</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>2.582</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>1.865</td>
<td>1.847</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>1.829</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>1.186</td>
<td>1.163</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>1.140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>0.855</td>
<td>0.850</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>0.845</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>0.555</td>
<td>0.556</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>0.577</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal F</td>
<td>C2</td>
<td>0.365</td>
<td>0.349</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>0.344</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont 1</td>
<td>G2</td>
<td>1.394</td>
<td>1.387</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>H2</td>
<td>1.380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pat 1</td>
<td>A3</td>
<td>1.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>1.091</td>
<td>1.078</td>
<td>1.21</td>
</tr>
</tbody>
</table>

*The represented in Example 1 and Figure 1 is for illustration only and should NOT be used in lieu of a dose response curve prepared with each assay.

11.0 G.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 0 μg/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

12.3 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Clinically, a DHEA-S value alone is not of diagnostic value and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the DHEA-S Accubind™ ELISA Test System are detailed in Table 1.

<table>
<thead>
<tr>
<th>Substances</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>100%</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.0003</td>
</tr>
<tr>
<td>Dehydrotestosterone</td>
<td>0.0008</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estriol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA-S Accubind™ Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Within Assay Precision (Values in μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The DHEA-S Accubind™ Microplate EIA Test System has a sensitivity of 0.042 μg/ml. The sensitivity was established by determining the 10 μg/ml serum calibrator and using the 2SD (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The DHEA-S Accubind™ Microplate EIA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and relatively high DHEA-S level populations were used. The %Cross Reactivity of the DHEA-S antibody to selected substances was evaluated by adding the interfering substance to the zero calibrator and using the 2SD (95% certainty) statistic to calculate the minimum dose.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Between Assay Precision (Values in μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
</tr>
</tbody>
</table>

15.0 REFERENCES

3. The within and between assay precision of the DHEA-S Accubind™ Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.