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** TEST PROCEDURE **

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 22°C).

1. Format the microplates: wells for each serum reference, control or specimen are treated in duplicate.

2. Pipette 0.010 ml (10 µl) of the appropriate serum reference, control or specimen into the assigned 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. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 350µl 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.

10. Add 0.100 ml (100µl) of working substrate solution to all wells and pipette assay blank (water) for control.

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

12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results should be recorded within thirty (30) minutes of adding the stop solution.

** PRECAUTIONS **

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 182 and HIV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and handled in the same way as other blood products. Follow the manufacturer's instruction for proper use. If a squeezing bottle is employed, fill each well by depressing the plunger (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

10. Add 0.100 ml (100µl) of substrate solution to all wells and pipette assay blank (water) for control.

Always reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results should be recorded within thirty (30) minutes of adding the stop solution.

** QUALITY CONTROL **

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These quality control levels should be determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supernatant. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be conserved with past experience. Significant deviation from established performance can indicate unacceptable test conditions or degradation of kit reagents. Fresh reagents should be used to determine the variation for the results.

** CALCULATION OF RESULTS **

A dose response curve is used to ascertain the concentration of DHEA-S in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate as used. Follow the manufacture's instruction for proper use. If a squeezing bottle is employed, fill each well by depressing the plunger (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

3. Add 0.100 ml (100µl) of substrate solution to all wells and pipette assay blank (water) for control.

4. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

5. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results should be recorded within thirty (30) minutes of adding the stop solution.

6. Dilute the contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (20-22°C) for up to 60 days.

7. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

8. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results should be recorded within thirty (30) minutes of adding the stop solution.

9. Dilute the samples suspected of concentrations higher than 8.0 µg/ml by 1:10 with DHEA-S 0 µg/ml calibrator or patient serum pools with a known low value for DHEA-S.

** 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 room temperature (20-22°C) for up to 60 days.

2. Working Substrate Solution - Stable for 1 year

Pour the contents of the vial labeled Solution A into the clear vial labeled Solution B. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

** REAGENT MATERI ALS PROVIDED **

A. DHEA-S Calibrators – 1ml/vial - Icon A

B. DHEA-S Enzyme Reagent – 6.0 ml/vial - Icon B

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

D. Wash Solution Concentrate – 20ml - Icon D

E. Substrate A – 7ml/vial - Icon E

F. Substrate B – 7ml/vial - Icon F

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** CALCULATION OF RESULTS **

A dose response curve is used to ascertain the concentration of DHEA-S in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
3. Connect the points with a best-fit curve.
4. To determine the concentration of DHEA-S for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient sample (1.078) intersects the dose response curve at (12.1 µg/ml) DHEA-S concentration (See Figure 1).

EXAMPLE 1

<table>
<thead>
<tr>
<th>ID</th>
<th>Sample</th>
<th>Number</th>
<th>Well</th>
<th>Abs A (µg/ml)</th>
<th>Mean</th>
<th>Abs B (µg/ml)</th>
<th>C.V.</th>
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<tbody>
<tr>
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<td>A1</td>
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<td>2.572</td>
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<td></td>
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<tr>
<td>B1</td>
<td>2.562</td>
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<tr>
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<tr>
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<tr>
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</tr>
</tbody>
</table>

*The above data and table below is for example only. Do not use it for calculating your results.

RISK ANALYSIS

A. ASSAY PERFORMANCE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Make sure that your instrumentation is properly calibrated according to MEDDEV Standards ISO 13485-2003 or equivalent.
9. All the instrumentation, used in the lab, should be properly maintained according to the manufacturer’s instructions.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield inaccurate results.

B. INTERPRETATION

1. 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.

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 I

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
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</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>1.0000</td>
</tr>
<tr>
<td>DHEA</td>
<td>0.0000</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.0003</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>0.0008</td>
</tr>
<tr>
<td>Cortisone</td>
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</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.00004</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estriol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estradiol</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>

D. SPECIFICITY

The % cross reactivity of the DHEA-S antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of DHEA-S needed to displace the same amount of labeled analog.

TABLE 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Dose Response Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>0.983</td>
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</tbody>
</table>

REFERENCES