3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

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

\[ \text{Ab}_\text{B} + \text{Ag} + \text{Antibody} \rightarrow \text{Ab}_\text{B} - \text{Ag} + \text{Antibody} \]

\[ \text{Ag} + \text{Antibody} \rightarrow \text{Ag} + \text{Antibody} \]

\[ K = \frac{[\text{Ab}_\text{B} - \text{Ag} + \text{Antibody}]}{[\text{Ag} + \text{Antibody}]} \]

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decapitation or aspiration.

\[ \text{Ag} + \text{Antibody} + \text{Streptavidin} \rightarrow \text{Streptavidin immobilized on well} \]

Two competitive reactions can be performed from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

A. DHEA-S Calibrators – 1ml/vial - Icons A-F

Six (6) vials of serum reference for DHEA-S at concentrations of 0.25, 1, 2, 5, 10, and 20 ng/mL in μg/mL. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (mM) by using 2.71 as the conversion.

For example: 1μg/mL x 2.71 = 2.71 μM

B. DHEA-S Tracer Reaction – 20ml – Icon

One (1) vial of DHEA-S (Analogue) horseradish peroxidase (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

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

One (1) bottle of reagent anti-DHEA-S biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservatives. Store at 2-8°C.

D. Light Reaction Wells – 96 wells - Icon

One (1) vial contains sufficient coated streptavidin in plastic microplate in a 96-well format with a blanking dye. Store at 2-8°C.

E. Wash Solution – 20ml – Icon

One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reactant A – 7.0ml/vial - Icon C

One (1) vial contains luminol in a buffer. Store at 2-8°C.

G. Signal Reactant B – 7.0ml/vial - Icon C

One (1) vial contains hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

H. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette capable of delivering 10μl and 50μl with a precision of better than 1.5%.
2. Dispensers for repetitive deliveries of 0.100 mL and 0.350 mL volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Vacuum aspirator (optional) for wash steps.
6. Areas for pipetting out (inert or non-reactive) for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can be universally used to test for all viruses, the individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from expected absorbance readings may indicate a change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C). **Test Procedure 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 foil strips or seal and store at 2-8°C.
2. Pipette 0.010 ml (10 µL) of the appropriate serum reference, control or patient specimen into the assigned well.
3. Add 0.050 ml (50µl) of the DHEA-S Enzyme Tracer to all wells.
4. Swit the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Anti-DHEA-S Biotin Reagent to all wells.
6. Swit 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 and centrifuge at 1000 x g for five (5) minutes.
9. Add 50µl of wash buffer (see Reagent Preparation Section), decap (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 bubble formation) to dispense the wash. Decant the wash and repeat four (4) additional times.
10. Add 100µl of developed solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood or serum or heparinized plasma in type and in all precautions in the collection of venipuncture samples. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use an EDTA blood tube(s) containing heparin. 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. Specimens( )cannot be assayed within this time, the specimen(s) may be stored at temperatures of 20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high concentration ranges. These controls should be treated as unknons and values determined in every test run. In the event of any discrepancies of female, male-female pairs, the laboratory should maintain 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 expected absorbance readings may indicate a change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature 20°C-27°C for up to 60 days.
11.0 QC. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
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.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the DHEA-S AccuLite™ CLIA Test System are detailed in Table 1. Expected Values for the DHEA-S Test System (μg/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.06 - 4.50</td>
<td>9.8%</td>
</tr>
<tr>
<td>Female</td>
<td>0.03 - 5.88</td>
<td>9.8%</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analyses using the method with a population indigenous to the area where the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA-S AccuLite™ CLIA 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 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>O</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>16</td>
<td>0.66</td>
<td>0.66</td>
<td>9.8%</td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>1.14</td>
<td>0.05</td>
<td>4.9%</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>4.87</td>
<td>0.21</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

For a complete description of the assay, see the complete instructions provided with the kit.

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>O</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>0.61</td>
<td>0.05</td>
<td>9.5%</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>1.36</td>
<td>0.34</td>
<td>3.1%</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>4.73</td>
<td>0.16</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

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

### Table 4

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Analysis Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>1.18</td>
<td>Y = 0.1440 0.9858(X)</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 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 5

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>0.0000</td>
</tr>
<tr>
<td>DHEA</td>
<td>0.0004</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.0003</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>0.0008</td>
</tr>
<tr>
<td>Cortisone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.0044</td>
</tr>
<tr>
<td>Spironolactone</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>

15.0 REFERENCES

10. Date: 0060412 DCO: 0642 Cat #: 5175-300