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:

$$k_{a} = \frac{K_{a} + Ag + Ab}{K_{a} + Ag + Ab + AbAg}$$

where $K_{a}$ is the dissociation constant.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Dehydroepiandrosterone (DHEA) is a C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. Due to the presence of a 17-oxo [rather than hydroxy] group, DHEA possesses relatively weak androgenic activity, which has been estimated at ~10% of that of testosterone. The physiologic role of DHEA is not well-defined. Since DHEA has a relatively low affinity constant for sex hormone binding globulin (SHBG), the bioactivity at the cell level maybe more significant than other androgenic steroids that have much higher affinity to SHBG. Abnormal levels have been reported in obesity and schizophrenia. Excessive DHEA secretion can cause acne, hirsutism and utilization. DHEA measurement is important in the investigation of adrenal androgen production for adrenal hyperplasia and tumors.

DHEA has a fast clearance turnover rate compared to its sulfated conjugate (Monobind Product 5125-300 DHEA-S). This leads to marked difference in circulation concentration compared to the free form, which has much longer half life. DHEA levels do circadian rhythm that reflects the secretion of ACTH and also varies during the menstrual cycle.

Measurement of serum DHEA is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism, while elevated levels occur in several conditions, including virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies and some cases of female hirsutism. Since very little DHEA is produced by the gonads, measurement of serum DHEA may aid in the localization of the androgen source in virilizing conditions.

The Monobind DHEA ELISA Kit uses a specific anti-DHEA antibody, and does not require prior 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 concentration permits the development of a linear relationship between concentration and absorbance, which is used for calibration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with DHEA concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

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 1&2 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 capable of causing disease. Good laboratory practice for handling blood products can be found in the Center for Disease Control / National Institutes of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

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 heparinised plasma in type and taken with the usual precautions in the collection of venipuncture samples. The blood should be collected in a red-top (or with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for 30 minutes at room temperature. Evacuated tube(s) containing heparin. Allow the blood to clot for 30 minutes at room temperature. After clot formation, aspirate the serum into a clean tube and centrifuge. Freeze and store at -20°C. Weigh the obtained serum samples into test tubes. Dilute the samples suspected of concentrations higher than the maximum working signal reagent, for example: 100 µl of 500 µg/ml diluted 1:5 with DHEA 0 ng/ml calibrator.

5.0 PRECAUTIONS

Do not use reagents beyond the kit expiration date. Always add reagents sho uld be used to determine the reason for the anticipated, within the above time constraint, pour the contents of A per two (2) eight well strips (A slight excess of solution is provided to two (2) eight well strips (A slight excess of solution is provided).

2.8.0 CALCULATION OF RESULTS

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 & 0.050 ml (25 & 50 µl) to provide precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350 ml (100 & 350 µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (20-500 µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Luminometer.
6. Absorbent Paper for blotting the microplate wells.
7. Cover and incubate for 60 minutes at room temperature.
8. Vacuum aspirator (optional) for wash steps.

9.0 TEST PROCEDURE

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

1. Format the microplates’ wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate and plate. Place and cover microwell strips into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference calibrator, control and plate. Replace and cover microwell strips into the aluminum bag, seal and store at 2-8°C.
3. Add 0.050 ml (50 µl) of the DHEA 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 Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 60 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 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (lap and blot) and 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 with depressing the content (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.
10. Add 0.100 ml (100 µl) of working signal reagent solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

11. Incubate at room temperature for five (5) minutes in the dark.
12. Swirl the microplate gently for 20-30 seconds to mix.
13. Read the relative light units (RLUs) in each well with a Microplate Luminometer.

Note: Dilute the samples suspected of concentrations higher than 50ng/ml 1:5 with DHEA ‘0’ ng/ml calibrator.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of progesterone in ng/ml on linear logarithmic graph paper. The laboratory should calibrate all reagents using standards of varying concentrations.

3.4. Add 0.100 ml (100 µl) of working signal reagent solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

11. Incubate at room temperature for five (5) minutes in the dark.
12. Swirl the microplate gently for 20-30 seconds to mix.
13. Read the relative light units (RLUs) in each well with a Microplate Luminometer.

Note: Computer data reduction software designed for CLIA Assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
1. The Dose Response Curve should be within established ranges. In order for the assay results to be considered valid the following Q.C. PARAMETERS must be met:

11. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from MonobindMonobind@monobind.com.

12.2 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 laboratory performance. Each laboratory should depend upon the assay, assay system, method and equipment used.

3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interference between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Biscotti LM, Stuart MC: Heterophilic antibodies: a problem for all immunoassays', Clin. Chem. 1986;32:37-33.) For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. 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.

6. 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.

7. Clinically, a DHEA 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 AccuLite® CLIA Test System are detailed within Table 2 and Table 3.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA 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.

15.0 REFERENCES


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