Access
Immunoassay Systems

DHEA-S

REF A10826

Intended Use

The Access DHEA-S assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of Dehydroepiandrosterone sulfate levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Dehydroepiandrosterone sulfate (DHEA-S) is a steriod synthesized primarily by the adrenal gland.[1] In those tissues containing sulfatase activity, DHEA-S can be converted to free steroid DHEA. Susbsequently, DHEA and/or DHEA-S may be partially metabolized into active androgens and estrogens.[1,2]

Serum and plasma DHEA-S levels are found to be the highest of all steroids. DHEA-S levels decrease with age in both men and women after maximum levels are reached around the third decade of life.[3] The half life for DHEA-S is approximately 8 to 10 hours as compared to the 30 to 60 minute half lives of other androgens.[1] The long half-life of serum DHEA-S coupled with the limited diurnal variation make DHEA-S a convenient marker for the assessment of adrenal production.[4]

DHEA-S may be used in the differential diagnosis of Cushing's syndrome.[5] DHEA-S may also be used to evaluate adrenocortical diseases, such as congenital adrenal hyperplasia and adrenal tumors.[6] In hirsute female patients, increased DHEA-S levels have been associated with virilizing adrenal tumors.[7] Patients with polycystic ovary syndrome have often demonstrated elevated levels of DHEA-S, suggesting an adrenal androgen contribution to the defect in this disorder.[8,9,10]

Principles of the Procedure

The Access DHEA-S assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-rabbit: rabbit anti-DHEA-S and DHEA-S alkaline phosphatase conjugate in TRIS-buffered protein solution. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of DHEA-S in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access DHEA-S Reagent Pack

Cat. No. A10826: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-rabbit IgG: rabbit anti DHEA-S in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	DHEA-S-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA matrix, < 0.1% sodium azide, and 0.1% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.1% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28–37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (lithium heparin, sodium heparin and EDTA) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[12]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Materials Provided

R1 Access DHEA-S Reagent Packs

Materials Required But Not Provided

1. Access DHEA-S Calibrators
   Provided at zero and approximately 20, 50, 200, 500 and 1000 µg/dL (0.54, 1.36, 5.43, 13.57 and 27.14 µmol/L).
   Cat. No. A10827
2. Quality Control (QC) materials: commercial control material
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793
   UniCel DxI Access Immunoassay Systems Wash Buffer II, Cat. No A79784 (Diluent pack for use with the UniCel DxI system onboard dilution feature).
5. Normal Saline

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use ten (10) µL of sample for each determination in addition to the sample container and system dead volumes. Use fifty (50) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is µg/dL. To change sample reporting units to the International System of Units (SI units), µmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply µg/dL by multiplication factor 0.02714.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access DHEA-S assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[13] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 2 –1000 µg/dL [0.05 – 27.14 µmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 2 µg/dL [< 0.05 µmol/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 850 µg/dL (23.07 µmol/L).
   - If a sample contains more than the stated value of the highest Access DHEA-S Calibrator (S5), report the result as greater than that value (i.e., > 1000 µg/ dL [> 27.14 µmol/L]). Alternatively, dilute one volume of sample with nine volumes of Wash Buffer II, Cat. No. A16792 (Access, Access 2, SYNCHRON LXi), Cat. No. A16793 (UniCel DxI) or normal saline. After assaying the diluted sample, multiply the calculated value by the dilution factor 10. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

     The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 9 volumes of UniCel DxI Access Immunoassay Systems Wash Buffer II, allowing samples to be quantitated up to approximately 10,000 µg/dL (271.4 µmol/L). The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[14,15]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access DHEA-S results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. DHEA-S was measured in human serum, heparinized plasma, and EDTA plasma samples from apparently healthy male and female subjects using the Access DHEA-S assay. The observed ranges of DHEA-S concentrations are shown below for each population represented.

Age (years)	n	Median† (µg/dL)	95% Reference Interval†† (µg/dL)
Females			
18–21	10	177	51–321
21–30	39	170	18–391
31–40	40	141	23–266
41–50	42	121	19–231
51–60	39	58	8–188
61–70	30	61	12–133
> 71	33	35	7–177
Males			
18–21	10	302	24–537
21–30	44	238	85–690
31–40	45	217	106–464
41–50	43	193	70–495
51–60	36	119	38–313
61–70	29	78	24–244
> 71	34	45	5–253

†Actual median of samples

††Based on parametric model for 2.5% to 97.5% reference interval

Specific Performance Characteristics

Methods Comparison

Representative data for methods comparison are provided for illustration only. Performance obtained in individual laboratories may vary.

A comparison of 263 values using the Access DHEA-S assay on the Access Immunoassay system and a commercially available immunoassay system gave the following statistical data using Deming calculations:

n	Range of Observations (µg/dL)	Intercept (µg/dL)	Slope	Correlation Coefficient (r)
263	15.3–966	6.6	1.028	0.993

Dilution Recovery (Linearity)

Representative data for linearity are provided for illustration only. Performance obtained in individual laboratories may vary.

Multiple dilutions of samples containing various DHEA-S levels with Wash Buffer II or normal saline resulted in the following data:

Dilutions with Wash Buffer II

Sample % Dilution	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	Recovery (%)
0.2	1.8	1.7	94.8
5	44.4	51.6	116.2
20	177.4	181.2	102.2
40	354.7	357.7	100.8
80	709.3	700.7	98.8
Neat	N/A	886.6	N/A
		Mean % Recovery	**102.6**

Dilutions with Normal Saline

Sample % Dilution	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	Recovery (%)
0.2	1.8	1.4	77.0
5	45.5	45.7	100.3
20	182.1	188.1	103.3
40	364.2	361.8	99.3
80	728.3	777.9	106.8
Neat	N/A	910.7	N/A
		Mean % Recovery	**97.4**

Spiking Recovery

Representative data for spiking recovery are provided for illustration only. Performance obtained in individual laboratories may vary.

Addition of six different levels of DHEA-S to a patient sample with low DHEA-S resulted in the following data:

Sample Level	Expected Concentration (µg/dL)	Determined Concentration (µg/dL)	Recovery (%)
1 (Neat)	N/A	14.3	N/A
2	32.8	34.2	104.3
3	112.8	114.6	101.6
4	212.8	218.4	102.6
5	412.8	426.3	103.3
6	612.8	637.3	104.0
7	812.8	887.5	109.2
		Mean % Recovery	**104.2**

Imprecision

Representative data for imprecision are provided for illustration only. Performance obtained in individual laboratories may vary.

This assay exhibits total imprecision of ≤ 10% at ≥ 20 µg/dL. One study, using commercially available human serum based control material generating a total of two assays, two replicates per assay, over 20 days provided the following data, analyzed via analysis of variance (ANOVA).[16]

Human Serum Control	Grand Mean (n=80) (µg/dL)	Within Run SD (µg/dL)	Within Run (%CV)	Total SD (µg/dL)	Total Imprecision (%CV)
Level 1	10.3	0.86	8.3	1.16	11.3
Level 2	34.4	1.10	3.2	1.76	5.1
Level 3	124.0	5.90	4.8	7.99	6.4
Level 4	347.3	8.92	2.6	15.38	4.4
Level 5	736.1	12.12	1.6	27.19	3.7

Analytical Specificity / Interferences

Representative data for analytical specificity/interferences are provided for illustration only. Performance obtained in individual laboratories may vary.

Samples containing up to 30.0 mg/dL (513 µmol/L) bilirubin, lipemic samples containing of equivalent of 1750 mg/dL (19.7 mmol/L) triglyceride, and hemolyzed samples containing up to 1000 mg/dL (10 g/L) hemoglobin do not affect the concentration of DHEA-S assayed. In

addition, 6.0 g/dL (60.0 g/L) human albumin added to endogenous albumin in the samples does not affect the concentration of DHEA-S assayed.

The following table lists substances that are similar in structure to DHEA-S.

These substances were tested at the concentrations indicated and found to have ≤ 1% cross-reactivity. DHEA had ≤ 0.5% cross-reactivity.

Substance	Analyte Added (µg/dL)
DHEA	4000
DHEA Glucuronide	5000
Aldosterone	5000
Androstenedione	1000
Androsterone	2000
Androsterone Glucuronide	5000
Cortisol	10,000
5-dihydrotestosterone	5000
Estradiol	5000
β-Estradiol-3-SO4-17-glucuronide	5000
Estriol	5000
Estrone	5000
Estrone-3-SO4	5000
19 Hydroxyandrostenedione	5000
Progesterone	5000
Testosterone	2000

Analytical Sensitivity

The lowest detectable level of DHEA-S distinguishable from zero (Access DHEA-S Calibrator S0) with 95% confidence is < 2 µg/dL (< 0.05 µmol/L). This value is determined by processing a complete six point calibration curve, controls, and 12 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is calculated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

Access
Immunoassay Systems

DHEA-S CALIBRATORS

REF A10827

Intended Use

The Access DHEA-S Calibrators are intended to calibrate the Access DHEA-S assay for the quantitative determination of Dehydroepiandrosterone sulfate levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access DHEA-S Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access DHEA-S Calibrators

Cat. No. A10827: S0–S5, 2.0 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Vial is stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

S0:	BSA matrix, < 0.1% sodium azide, and 0.5% ProClin** 300. Contains 0.0 µg/dL (µmol/L) DHEA-S.
S1, S2, S3, S4, S5:	DHEA-S in BSA matrix at approximate levels 20, 50, 200, 500, and 1000 µg/dL (0.54, 1.36, 5.43, 13.57 and 27.14 µmol/L), respectively with < 0.1% sodium azide, and 0.5% ProClin 300.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi. Irritant: 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28–37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access DHEA-S Calibrators are provided at six levels - zero and approximately 20, 50, 200, 500 and 1000 µg/dL prepared gravimetrically from crystalline DHEA-S in BSA based matrix. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

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