en

Immunoassay

REF **CMF0701/CMF0702/CMF0703/CMF0704/CMF0705**

50 tests*1 /100 tests*1 /100 tests*2 /100 tests*5 /50 tests*2

DHEA-S CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of DHEA-S (Dehydroepiandrosterone sulfate) in human serum.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact your local dealer for all product-related questions in your local language.

Introduction

Dehydroepiandrosterone sulfate (DHEA-S), also known as prasterone sulfate, is a naturally occurring, endogenous androstane steroid and neurosteroid and the 3β-sulfate ester of dehydroepiandrosterone (DHEA). As the sodium salt, prasterone sodium sulfate, DHEA-S is used as a pharmaceutical drug in Japan in the treatment of insufficient cervical ripening and cervical dilation during childbirth.[1-7]
Dehydroepiandrosterone sulfate levels above 1890 micromol/L or 700-800 μg/dL are highly suggestive of adrenal dysfunction because DHEA-S is made exclusively by the adrenal glands.[8,9] Presence of DHEA-S is therefore used to rule out ovarian or testicular origin of excess androgen.

Measurement Principle

This assay is based upon the one-step competitive method. The secondary antibody coated microparticles and rabbit polyclonal antibody-linked antibody solution are added, antibodies are generated after they bind together, then DHEA-S present in the sample and DHEA-S antigen in the Enzyme Conjugate are added and complete to bind to the antibodies. After the washing, a complex is generated among the antibodies, the DHEA-S within the sample and enzyme-linked antigens by immunological reactions. Chemiluminescent Substrate are then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is reversely proportional to the concentration of DHEA-S in the patient sample.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is PBS-K buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.
Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2
Antibody Solution	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Goat anti-rabbit antibody coated microparticles in PBS buffer containing BSA (bovine serum albumin). Contains ProClin 300® and sodium azide preservatives.

- Enzyme Conjugate

HRP (horseradish peroxidase) labeled DHEA-S in PBS buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

- Antibody Solution

Rabbit polyclonal antibody in PBS buffer containing BSA (bovine serum albumin). Contains ProClin 300® preservative.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The analyte in the 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 reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. When storing the calibrators, be certain the vials are securely sealed.
14. Ensure the microparticles are resuspended before loading it on the analyzer.
15. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
16. Do not substitute any reagent in this kit from other manufacturers or other lots.
17. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored

as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10 ℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 60 days.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot or red blood cells.
6. Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes or cups on the sample rack, 25 μL of samples and calibrators for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Enzyme Conjugate and Antibody Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of DHEA-S in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tubes or cups and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a DHEA-S value exceeding 1000 μg/dL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

- The concentration of the sample after dilution should not be less than 150 μg/dL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of DHEA-S in the samples is determined from the measured light production by means of the stored calibration data. Refer

to the Assay Analyzer's operation manual on stored data.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Performance of this test has not been established with neonatal samples.
4. This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
5. This test measures concentrations within the range of 2-1000 μg/dL. If DHEA-S concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:4 of this test, allowing samples to be quantitated up approximately to 5000 μg/dL.

Biological Reference Interval

A study of an apparent normal adult population was undertaken to determine reference intervals for this assay, with 5% and 95% as limit using percentile method. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Gender	Age	Sample no.	Reference interval (μg/dL)
Females	18-20	101	75-314
	21-30	109	30-361
	31-40	120	30-263
	41-50	115	36-248
	51-60	98	10-185
	61-70	96	16-139
	>70	91	4-149
Males	18-20	135	34-530
	21-30	164	91-612
	31-40	105	118-420
	41-50	124	71-437
	51-60	102	51-296
	61-70	93	37-218
	>70	90	10-215
Children	<1 week	110	108-603
	1-4 week	105	32-428
	1-12 month	108	5-122
	1-4	116	1-19
	5-9	118	3-85
	10-17	108	27-447

Performance Characteristics

1. Measurement Precision

This assay is designed to have a within-run precision of <10%. 2 human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision SD	%CV
1	1	10	22.60	1.31	5.79
2	1	10	475.10	14.34	3.02

This assay is designed to have a between-run precision of <15%. 2 human serum based panel members (1 and 2) were assayed, using 1 batch of reagents, in replicates of 10, once per day across 3 testing days. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Between-run Precision SD	%CV
1	1	30	22.90	1.48	6.47
2	1	30	473.23	13.85	2.93

2. Analytical Sensitivity

Analytical sensitivity, defined as the concentration corresponding to the mean RLUs of 20 replicates of calibrator S0 minus 2 standard deviations, is ≤2 μg/dL.

3. Analytical Specificity

Cross reaction: This assay is designed to have an analytical specificity less than 3 μg/dL cross reactivity with the substances listed below, at the concentration levels listed, in calibrator diluent.

Substance	Concentration (μg/dL)	Measured Value (μg/dL)
DHEA	4000	<3
Aldosterone	5000	<3
Progesterone	5000	<3
Estriol	5000	<3
E2	5000	<3
17α-hydroxypregnenolone	1000	<3
Prednison	1000	<3
Testosterone	2000	<3
Cortisone	1000	<3
Cortisol	10000	<3
Hexadecadrol	1000	<3
21-Hydroxyprogesterone	1000	<3
Dehydrocorticosterone	1000	<3

Interference: No interference with 1.25 g/L of haemoglobin, 0.1 g/L of Bilirubin, 30 g/L of Triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and a DHEA-S Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	200	0.1391	0.9979	0.9452

Literature References

1. J. Elks (14 November 2014). The Dictionary of Drugs: Chemical Data: Chemical Data, Structures and Bibliographies. Springer. pp. 641.
2. John W. Blunt; Murray H. G. Munro (19 September 2007). Dictionary of Marine Natural Products with CD-ROM. CRC Press. pp. 1075.
3. A. Kleemann; J. Engel; B. Kutscher; D. Reichert (14 May 2014). Pharmaceutical Substances, 5th Edition, 2009: Syntheses, Patents and Applications of the most relevant APIs. Thieme. pp. 2441–2442.
4. Martin Negwer; Hans-Georg Scharnow (2001). Organic-chemical drugs and their synonyms: (an international survey). Wiley-VCH. p. 1831.
5. Jianqiu, Y. (1992). Clinical Application of Prasterone Sodium Sulfate. Chinese Journal of New Drugs, 5, 015.
6. Sakaguchi M, Sakai T, Adachi Y, Kawashima T, Awata N (1992). "The biological fate of sodium prasterone sulfate after vaginal administration. I. Absorption and excretion in rats". J. Pharmacobio-dyn. 15 (2): 67–73.
7. Sakai, T., Sakaguchi, M., Adachi, Y., Kawashima, T., & Awata, N. (1992). The Biological Fate of Sodium Prasterone Sulfate after Vaginal Administration II: Distribution after Single and Multiple Administration to Pregnant Rats.
8. Somani N, Harrison S, Bergfeld WF (2008). "The clinical evaluation of hirsutism". Dermatologic therapy. 21 (5): 376–91.
9. "Polycystic Ovarian Syndrome Workup". eMedicine. 25 October 2011. Retrieved 19 November 2011.