Diagnostics Biochem Canada

5α-Androstane-3α,17β Diol Glucuronide (3α-Diol G) ELISA

REF CAN-DG-460

ASSAY PROCEDURE

- Bring kit components to room temperature.
- Prepare working solutions.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the conjugate working solution into each well.
- Incubate on a plate shaker for 30 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of stopping solution.
- Read the plate on a microplate reader at 450 nm.

5α-Androstane-3α, 17β-diol glucuronide is a C19 steroid and is either abbreviated as 3α-Diol G, 5α-Diol G or simply, α-Diol G. It is produced mainly as a metabolite of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3α-Diol G leads to excessive hair formation, notably where hair is not normally present in women.

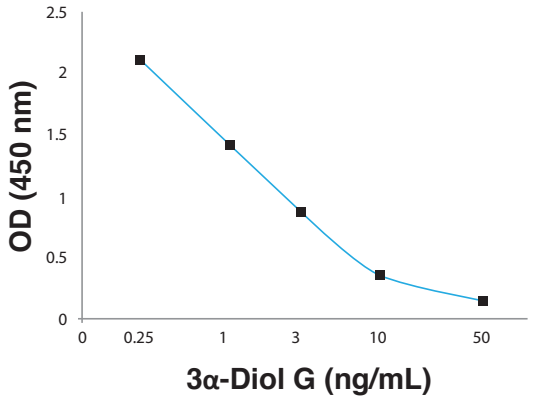
In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism.

Among the steroids known to be precursors for 3α-Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3α-Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovarian syndrome (PCO). 3α-Diol G determinations have therefore proved to be a useful indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with PCO.

Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3α-Diol G levels, a side effect resulting in the appearance of hair in previously hairless areas.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of 3α-Diol G in the sample. A set of standards is used to plot a standard curve from which the amount of 3α-Diol G in patient samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DBC Direct 3α-Diol G ELISA kit is **0.1 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct 3α-Diol G ELISA kit with 3α-Diol G cross-reacting at 100%.

Compound	% Cross-Reactivity
3α-Diol G	100
Testosterone	0.2
Progesterone	0.16
Androstenedione	0.14
Cortisol	0.05

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epi-androsterone, 17β-Estradiol and Estrone.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.87	0.07	7.8
2	6.86	0.49	7.2
3	21.26	1.29	6.0

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.98	0.10	10.4
2	7.05	0.46	6.5
3	20.92	2.26	10.8

RECOVERY

Spiked samples were prepared by adding defined amounts of 3α-Diol G to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Results	Recovery %
1 Unspiked	0.67	-	-
+ 0.5	1.07	1.17	91.4
+ 5.0	4.99	5.67	88.0
+ 15.0	12.66	15.67	80.8
2 Unspiked	1.83	-	-
+ 0.5	2.07	2.33	88.8
+ 5.0	6.18	6.83	90.5
+ 15.0	17.64	16.83	104.8
3 Unspiked	12.76	-	-
+ 0.5	15.32	13.26	115.5
+ 5.0	19.22	17.76	108.2
+ 15.0	22.68	27.76	81.7

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	6.24	-	-
1:2	2.83	3.12	90.7
1:4	1.55	1.56	99.4
1:8	0.74	0.78	94.9
2	13.55	-	-
1:2	6.00	6.77	88.6
1:4	2.71	3.39	80.0
1:8	1.70	1.64	103.6
3	17.05	-	-
1:2	6.93	8.53	81.2
1:4	4.09	4.26	96.0
1:8	2.34	2.13	109.8

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Males	1.53–14.82
Females Premenopausal	0.22–4.64
Postmenopausal	0.61–3.71
Puberty	0.51–4.03

Ordering Information: REF CAN-DG-460

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