

Read entire protocol before use.

3 α -Diol G-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human 5 α -Androstane-3 α -17 β -Diol-Glucuronide (3 α -Diol G) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **3 α -Diol G-RIA** -CT Kit
B. Catalogue number : **BL-33-CT**: 100 tests
C. Manufactured by : Bio-Line S.A.
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III. CLINICAL BACKGROUND

5 α -Androstane-3 α -17 β -Diol-Glucuronide (3 α -Diol G) is a C19 steroid. 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 the 3 α -Diol G, leads to excessive hair formation – notably conspicuous 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 3 α -Diol G, namely dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), DHT androstenedione and testosterone, only 3 α -Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovaries (PCO).


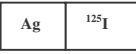
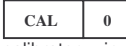
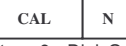

3 α -Diol G determinations have therefore proved to be useful as an indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with polycystic ovaries.

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

IV. PRINCIPLES OF THE METHOD

A fixed amount of ¹²⁵I labelled 3α-Diol G competes with the 3α-Diol G to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography are required because of the high specificity of the coated antibodies. After a 2 hours incubation at RT on a shaker, an aspiration step terminates the competition reaction. The tubes are then washed twice with 2 ml of Working Wash Solution and aspirated again. A calibration curve is plotted and the 3α-Diol G concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

| Reagents | 100 Test Kit | Colour Code | Reconstitution |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|-------------|------------------------------------------------------------|
|  Tubes coated with anti 3α-Diol G | 2 x 50 | grey | Ready for use |
|  TRACER: ¹²⁵ Iodine labelled 3α-Diol G (HPLC grade) in buffer with bovine casein (1%) and azide (<0.1%) | 1 vial 53 ml 185 kBq | Red | Ready for use |
|  Zero calibrator in bovine serum, thymol (<0.1%) and gentamycin (< 0.1%) | 1 vial lyophilized | yellow | Add 2 ml distilled water |
|  Calibrators 3α-Diol G - N = 1 to 5 (see exact values on vial labels) in bovine serum, thymol (<0.1%) and gentamycin (<0.1%) | 5 vials lyophilized | yellow | Add 0.5 ml distilled water |
| WASH SOLN CONC Wash solution (Phosphate Buffer) | 1 vial 50 ml | brown | Dilute 25 x with distilled water (use a magnetic stirrer). |
|  Controls - N = 1 or 2 in human serum, thymol (<0.1%) and gentamycin (<0.1%) | 2 vials lyophilized | silver | Add 0.5 ml distilled water |

Note : Use the zero calibrator for sample dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- Distilled water
- Pipettes for delivery of: 100 µl, 500 µl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Tubes shaker
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional)
- Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Calibrators** : Reconstitute the zero calibrator with 2.0 ml distilled water and other calibrators with 0.5 ml distilled water.
- Controls** : Reconstitute the controls with 0.5 ml distilled water.
- Working Wash solution** : Prepare an adequate volume of Working Wash solution by adding 24 volumes of distilled water to 1 volume of Wash Solution (25x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for one week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma samples must be kept at 2-8°C.
- If the test is not run within 24 hrs., storage at -20°C is recommended.
- Avoid successive freezing and thawing.
- Heparinized plasma yields 15 % lower results than serum :
Y (Hep. plasma) = 0.86 x (serum) + 0.52 r = 0.96 n = 13
- EDTA plasma yields 25 % lower results than serum :
Y (EDTA plasma) = 0.74 x (serum) + 0.52 r = 0.97 n = 13

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a standard curve for each run, do not use data from previous runs.

B. Procedure

- Label coated tubes in duplicate for each calibrator, sample, control. For the determination of total counts, label 2 normal tubes
- Briefly vortex calibrators, samples and controls and dispense 100µl of each into respective tubes.
- Dispense 0.5 ml of ¹²⁵Iodine labelled 3α-Diol G into each tube, including the uncoated tubes for total counts.
- Shake the tube rack gently.
- Incubate for 2 hours at room temperature with continuous shaking.
- Aspirate the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
- Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate. Avoid foaming during the addition of the Working Wash solution.
- Wash tubes again with 2 ml Working Wash solution (except total counts) and aspirate.
- After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations, reject obvious outliers.
- Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the 3α-Diol G concentration of each calibrator point.
- Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is to be used with results, "4 parameters" function curve fitting is recommended.
- By interpolation of the sample (B/B₀(%)) values, determine the 3α-Diol G concentrations of the samples from the reference curve.
- For each assay, the percentage of total tracer bound in the absence of unlabelled 3α-Diol G (B₀/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

| 3 α -Diol G | cpm | B/Bo (%) |
|--------------------|-------|----------|
| Total count | 35967 | |
| Calibrator | | |
| 0.0 ng/ml | 13912 | 100.0 |
| 0.2 ng/ml | 11443 | 82.2 |
| 1.2 ng/ml | 8385 | 60.3 |
| 6.0 ng/ml | 4611 | 33.1 |
| 25.0 ng/ml | 1796 | 12.9 |
| 75.0 ng/ml | 960 | 6.9 |

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.05 ng/ml.

B. Specificity

| Compound | Cross-Reactivity (%) |
|------------------------------------------------------------------|----------------------|
| 5 α -Androstane-3 α -17 β -diolG | 100.00 |
| 5 α -Androstane-3 α -17 β -diol | 10.69 |
| 5 α -Androstane-3 α -17 β -diol-3Glucuronide | 5.86 |
| 5 α -Dihydrotestosterone Glucuronide | 1.75 |
| Progesterone | 0.03 |
| Testosterone glucuronide | 0 |
| Testosterone | 0 |
| 11 β -hydroxytestosterone | 0 |
| 5 α -Dihydrotestosterone | 0 |
| 5 β -Dihydrotestosterone | 0 |
| Cortisol | 0 |
| Dehydroepiandrosterone | 0 |
| Estrone | 0 |
| Androstenedione | 0 |
| 5-Androstene-3 β -17 β -diol | 0 |
| 17 β -Estradiol | 0 |

Note :this table shows the cross-reactivity for the anti 3 α -Diol G.

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

| Serum | N | <X> \pm SD (ng/ml) | CV (%) | Serum | N | <X> \pm SD (ng/ml) | CV (%) |
|-------|----|----------------------|--------|-------|----|----------------------|--------|
| A | 20 | 2.64 \pm 0.15 | 5.7 | A | 10 | 2.76 \pm 0.17 | 6.4 |
| B | 20 | 10.11 \pm 0.50 | 4.9 | B | 10 | 10.32 \pm 0.70 | 7.2 |

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

| Sample | Dilution | Theoretical Concent. (ng/ml) | Measured Concent. (ng/ml) |
|--------|----------|------------------------------|---------------------------|
| Serum | 1/1 | 50.70 | 50.70 |
| | 1/2 | 25.35 | 24.58 |
| | 1/4 | 12.67 | 11.96 |
| | 1/8 | 6.33 | 6.49 |
| | 1/16 | 3.16 | 3.26 |
| | 1/32 | 1.58 | 1.48 |
| | 1/64 | 0.79 | 0.62 |

Samples were diluted with the zero calibrator.

RECOVERY TEST

| Sample | added 3 α -Diol G (ng/ml) | Recovered 3 α -Diol G (ng/ml) | Recovered (%) |
|--------|----------------------------------|--------------------------------------|---------------|
| Serum | 50 | 48.4 | 96.8 |
| | 20 | 21.3 | 106.0 |
| | 10 | 9.5 | 95.0 |
| | 5 | 4.9 | 98.0 |

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 20 minutes after the calibrator has been added to coated tubes.

TIME DELAY

| Serum (ng/ml) | 0' | 20' |
|---------------|------|------|
| Serum 1 | 2.92 | 2.90 |
| Serum 2 | 9.60 | 9.59 |

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Do not freeze-thaw more than twice.
- To the best of our knowledge, no international reference material exists for this parameter.

XV. REFERENCE INTERVALS

| Population | Absolute range (ng/ml) | Median (ng/ml) | |
|------------|------------------------|----------------|-----|
| Female | Premenopausal | 0.3 - 7.9 | 1.9 |
| | Postmenopausal | 0.1 - 5.9 | 1.5 |
| | Hirsute | 1.6 - 9.3 | 4.6 |
| Male | | 1.0 - 23.6 | 6.4 |

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious. Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves. All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiosafety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

XVII. BIBLIOGRAPHY

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Assesment of 5 α -reductase activity in hirsute women: comparison of serum androstanediol glucuronide with urinary androsterone and aetiocholanolone excretion.
Clinical Endocrinology, 40, 459-464.
6. THOMPSON D.L. *et al.* (1990)
Androsterone Glucuronide is a marker of adrenal hyperandrogenism in hirsute women.
Clinical Endocrinology, 32, 283-292.

XVIII. SUMMARY OF THE PROTOCOL

| | TOTAL COUNTS μ l | CALIBRATORS μ l | SAMPLE(S) CONTROLS μ l |
|-----------------------|---------------------------------------|------------------------|-------------------------------|
| Calibrators (0 to 5) | - | 100 | - |
| Samples, controls | - | - | 100 |
| Tracer | 500 | 500 | 500 |
| Incubation | 2 hours at RT with continuous shaking | | |
| Separation | - | aspirate | |
| Working Wash solution | | 2.0 ml | |
| Separation | | aspirate | |
| Working Wash solution | | 2.0 ml | |
| Separation | | aspirate | |
| Counting | Count tubes for 60 seconds | | |

| | | |
|-------------------------------------|-----------------------|---------------------------|
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