1. Users should have a thorough understanding of this protocol.

2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.

3. When the use of water is specified for dilution or reaction, use deionized or distilled water.

4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.

5. All kit reagents and specimens should be brought to room temperature and mixed according to the label before use. Avoid repeated freezing and thawing of reagents and specimens.

6. A calibrator curve must be established for every run.

7. The controls should be included in every run and fall within established confidence limits.

8. In proper procedural techniques, imprecision pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.

9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.

10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.

11. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.

12. A pipette tip should be used for dispensing each reagent, sample, standard, control, and calibrator.

13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date.

2. All the reagents within the kit are calibrated for the determination of 3α-Diol G in saliva, plasma or other suitable body fluids.

3. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.

4. Any samples or control sera containing azide or thimerosal may not be compatible with this kit, as they may lead to false results.

5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the kit has been demonstrated to be highly sensitive to the presence of 3α-Diol G in human serum. However, the presence of 3α-Diol G in serum is not a specific indicator of any disease process, but rather reflects the presence of a specific metabolite of cholesterol metabolism.

6. Only the 240 µL of conjugate concentrate in 12 mL of assay buffer should be used to dilute the 240 µL of conjugate concentrate in 12 mL of lysis buffer. Discard any that is left over.

7. This assay is a direct system; no specimen pretreatment is necessary.

8. Rabbit Anti-3α-Diol G Antibody—Coated Break-Apart Well Microplate — Ready to Use

9. Preparation of conjugate working solution: Dilute conjugate concentrate 1:10 in distilled or deionized water before use. If the whole plate is to be used, dilute 50 mL of wash buffer concentrate in 450 mL of water.

10. Only calibrator A may be used to dilute any high serum specimens. The use of any other reagent may lead to false results.

11. LIA Substrate Reagent A — Requires Preparation

12. LIA Substrate Reagent B — Requires Preparation

13. LIA Substrate Reagent C — Requires Preparation

14. LIA Substrate Reagent D — Requires Preparation

15. LIA Substrate Reagent E — Requires Preparation

16. LIA Substrate Reagent F — Requires Preparation

17. Preparations of conjugate working solution: Dilute conjugate concentrate 1:10 in distilled or deionized water before use. If the whole plate is to be used, dilute 50 mL of wash buffer concentrate in 450 mL of water.


20. Preparation: See preparation of LIA working substrate solution.


22. Preparation: See preparation of LIA working substrate solution.

23. Preparation: See preparation of LIA working substrate solution.
PREPARATION OF LIA WORKING SUBSTRATE SOLUTION
Mix 1 part of LIA substrate reagent A with 2 parts of LIA substrate reagent B and dilute this mixture 1:3.3 with LIA substrate reagent C. This gives the ready to use substrate solution. Prepare fresh for each use.
If the whole plate is to be used prepare working substrate solution as follows: Combine 1 mL of LIA substrate reagent A with 2 mL of LIA substrate reagent B. To the 3 mL of this mixture add 10 mL of LIA substrate reagent C.
Total volume = 13 mL of working substrate solution.
Stability: Working substrate solution is stable for 24 hours at room temperature.

ASSAY PROCEDURE
Important Notes:
1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is completed without interruption to ensure equal volumes of wash are used in each well. (We recommend using a multichannel pipette.)
4. Pipette 50 μL of each calibrator, control and sample stock into correspondingly labelled wells in duplicate.
5. Incubate on a plate shaker (approximately 200 rpm) at room temperature without shaking.
6. Wash the wells 5 times with 300 μL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 100 μL of LIA working substrate solution into each well. (We recommend using a multichannel pipette.)
8. Shake for 5 seconds. Incubate for 10–30 minutes at room temperature without shaking.
9. Measure the RLU in each well on a microplate luminometer.
10. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
11. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.

CALCULATIONS
5. If a sample reads more than 50 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA*
Sample data only. Do not use to calculate results.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>RLU 1</th>
<th>RLU 2</th>
<th>Mean RLU</th>
<th>RLU/RULSUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 0 ng/mL</td>
<td>1914</td>
<td>1917</td>
<td>1916</td>
<td>100</td>
</tr>
<tr>
<td>B, 0.25 ng/mL</td>
<td>1669</td>
<td>1663</td>
<td>1667</td>
<td>87</td>
</tr>
<tr>
<td>C, 0.25 ng/mL</td>
<td>1181</td>
<td>1169</td>
<td>1170</td>
<td>61</td>
</tr>
<tr>
<td>D, 3 ng/mL</td>
<td>693.8</td>
<td>677.1</td>
<td>685.4</td>
<td>36</td>
</tr>
<tr>
<td>E, 5 ng/mL</td>
<td>280.1</td>
<td>296.0</td>
<td>288.0</td>
<td>15</td>
</tr>
<tr>
<td>F, 50 ng/mL</td>
<td>84.84</td>
<td>83.32</td>
<td>84.01</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* It is recommended to use the RLU/RULSUM values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show marked differences in values, however, the RLU/RULSUM values remain consistent.

TYPICAL CALIBRATION CURVE
Sample curve only. Do not use to calculate results.

PERFORMANCE CHARACTERISTICS
SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DBC 3α-Diol G LIA kit is 0.1 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)
The following compounds were tested for cross-reactivity with the DBC 3α-Diol G LIA kit with 3α-Diol G cross-reacting at 100%:

<table>
<thead>
<tr>
<th>Steroid</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3α-Diol G</td>
<td>100</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.2</td>
</tr>
<tr>
<td>Progestosterone</td>
<td>0.16</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.16</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epistane, Androstosterone, T3 and Estrone.

REFERENCES

OTHER RELATED DBC KITS
Also available from stock are the following DBC kits: 3α-Diol G ELISA Kit, REF: CAN-DG-460 DHT ELISA Kit, REF: CAN-DHT-280

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3α-Diol G</td>
<td>1.7</td>
<td>98.1</td>
</tr>
<tr>
<td>2</td>
<td>3α-Diol G</td>
<td>2.6</td>
<td>102.4</td>
</tr>
<tr>
<td>3</td>
<td>3α-Diol G</td>
<td>25.4</td>
<td>98.3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.57</td>
<td>0.10</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>8.86</td>
<td>0.32</td>
<td>3.58</td>
</tr>
<tr>
<td>3</td>
<td>10.49</td>
<td>0.87</td>
<td>8.34</td>
</tr>
</tbody>
</table>

EXPECTATION NORMAL VALUES
As for all clinical assays each laboratory should conduct data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>1.53–14.82</td>
</tr>
<tr>
<td>Females</td>
<td>2.02–4.46</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>0.61–3.71</td>
</tr>
<tr>
<td>Puberty (Female)</td>
<td>0.54–4.03</td>
</tr>
</tbody>
</table>

EMERGO EUROPE
Prinssegracht 20
2514 AP The Hague
The Netherlands

SYMBOLS
- www.dbc-labs.com
- Tel: (519) 681-8734
- 384 Neptune Crescent
- London, Ontario, Canada N6M 1A1
- Fax: (519) 681-8734
- e-mail: dbc@dbc-labs.com
- An ISO 13485 Registered Company

DBC-Bioschem Biochem Canada Inc.
384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: (519) 681-8734
Fax: (519) 681-8734
e-mail: dbc@dbc-labs.com
www.dbc-labs.com
An ISO 13485 Registered Company