

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION CHECKLIST

- I. 510(k) Number:** K030730
- II. Analyte:** Testosterone
- III. Type of test:** Quantitative
- IV. Applicant Name:** Diagnostic Biochem Canada, Inc.
- V. Proprietary and Established Names:** Direct ELISA Kit, Free Testosterone
- VI. Regulatory Information:**
- A. Regulation section: CFR 862.1680 Testosterone
 - B. Classification: Class 1
 - C. Product Code: CDZ
 - D. Panel: Chemistry
- VII. Intended Use (per labeling):** For the direct determination of Free testosterone by EIA in serum.
- A. Special Instrument Requirements: Automatic plate washer and microwell plate reader with a filter set at 450 nm
 - B. Special condition for use statement(s): None
 - C. Indication for use: **The dbc CAN-ftE-260 EiAsy Free Testosterone enzymeimmunoassay (EIA) kit provides the reagents necessary for the direct determination of Free Testosterone in human serum. This assay is intended for in vitro diagnostic use only. Measurement of Free Testosterone are used in the diagnosis in male sex hormones (androgens) and in female hirsutism (excessive hair) and virilisation (masculinization).**
- V. Device description:** The kit consists of a microwell plate, conjugate, set of 6 standards (0-100 pg/mL), a control, 2 buffers, a substrate, and stopping solution.
- VI. Substantial Equivalence Information:**
- A. Predicate Device(s): Coat-A-Count Free Testosterone kit, DPC
 - B. Predicate K number(s): k844423
 - C. Comparison with predicate: Both products are immunoassays involving microplate technology. The products have the same intended use.
 - D. Standard/Guidance document referenced (if applicable): Not applicable.

VII. Test Principle: This is a competitive immunoassay where free testosterone and enzyme labeled testosterone compete for the limited binding sites on testosterone specific-antibody (polyclonal) immobilized on microtiter plate wells. Unbound testosterone is washed away, substrate is added. The color developed is measured by a microplate reader at 450 nm. Unknowns are calculated using a standard curve.

VIII. Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. *Precision (intra-assay)/Reproducibility:* 10 replicates of three levels of control sera (1.2, 15.9, and 62.4 pg/mL) were assayed 10 times. CVs were 17, 4.9, and 4.7 percent, respectively. Inter-assay precision was calculated by running 10 replicates of three levels of control sera (1.0, 25.8, and 75.8 pg/mL) were assayed over two weeks. CVs were 12.4, 5.3, and 8.8 percent, respectively.
2. *Linearity/assay reportable range:* The linearity of the assay is supported by the span of the calibrators.
3. *Traceability/controls:* The control is pooled serum, and its value is assigned by replicate measurements with the test system.
4. *Detection limit (functional sensitivity):* 10 replicates of the zero calibrator were assayed. The mean plus 2 standard deviations was found to be 0.167 pg/mL which was read from a standard curve.
5. *Analytical Specificity:* Percent cross-reactivity was evaluated on 16 structurally similar compounds. Testosterone was 100%, 5-dihydrotestosterone was 5.2% and androstenedione was 1.4 % cross-reactive. The remainder of the compounds were less than 1% cross-reactive.

B. Comparison studies:

1. *Matrix comparison:* Not applicable.
2. *Method comparison:* 64 samples were evaluated by the candidate device and the DPC Coat-A-Count Free Testosterone assay. The regression analysis reveals: $Y = 1.1036 \text{ (DPC)} + 0.76 \text{ pg/mL}$, $R = 0.89$. Of note is the fact that several data pairs exhibit some significant differences compared to predicate device results, some of which cause a difference in interpretation, i.e., by one method the results fall within the expected range and by the other device they fall outside the expected range. For example: 11.4 and 17.4, 24.1 and 30.8, 12.0 and 17.6, 0.4 and 2.9, and 0.26 and 5.2 pg/mL. The sponsor states that this is due to the different antibodies and tracer conjugates used, which result in differences in specificity and susceptibility to interferences.
3. *Clinical sensitivity:* Not demonstrated.
4. *Clinical specificity:* Not demonstrated.

C. Cut-off: Not applicable.

- D. Expected values/Reference range: Forty-three apparently healthy females and forty-five apparently healthy males were evaluated in a reference range study. The 95% confidence range for females was found to be 0.04-6.04 pg/mL and the range for males was 4.23-30.10 pg/mL.

- IX. Other supportive information :** The sponsor evaluated whether the conjugate binds to SHBG (sex hormone binding globulin). Charcoal-stripped human serum was spiked with SHBG at concentrations ranging from 6 to 200 micrograms/mL and assayed. All concentrations exhibited less than 10% binding.

To investigate whether HSA (Human Serum Albumin) effects the assay HSA was added in concentrations of 1.25, 2.5, and 5.0 grams to three patient samples previously assayed. Results of the study follow:

Albumin added (in g/dL) versus measured testosterone levels (in pg/mL)

Sample	0 HAS	1.25 HAS	2.5 HAS	5.0 HAS
1	0.52	0.34	0.54	0.53
2	15.8	14.2	12.5	11.0
3	26.2	23.0	21	18.6

- X. Conclusion: Substantially Equivalent**