

**ADVIA Centaur® XP**  
**ADVIA Centaur® XPT**  
Immunoassay Systems

## Testosterone II (TSTII)

|                                        |                                                                            |            |                      |
|----------------------------------------|----------------------------------------------------------------------------|------------|----------------------|
| Current revision and date <sup>a</sup> | Rev. 09, 2023-03                                                           |            |                      |
| Product Name                           | ADVIA Centaur® Testosterone II (100 tests)                                 | <b>REF</b> | 10696862             |
|                                        | ADVIA Centaur Testosterone II (500 tests)                                  | <b>REF</b> | 10696863             |
| Systems                                | ADVIA Centaur XP system<br>ADVIA Centaur XPT system                        |            |                      |
| Materials Required but Not Provided    | ADVIA Centaur Ancillary Probe Wash 1                                       | <b>REF</b> | 03395373             |
|                                        | ADVIA Centaur Probe Wash 4                                                 | <b>REF</b> | 04750940             |
|                                        | ADVIA Centaur Wash 1 (2 x 1500 mL)                                         | <b>REF</b> | 01137199<br>(112351) |
|                                        | ADVIA Centaur Wash 1 (2 x 2500 mL)                                         | <b>REF</b> | 03773025             |
| Specimen Types                         | Human serum and plasma (lithium heparin, sodium heparin, dipotassium EDTA) |            |                      |
| Assay Range                            | 7.00–1500.00 ng/dL (0.24–52.05 nmol/L)                                     |            |                      |
| Reagent Storage                        | 2–8°C                                                                      |            |                      |
| Reagent On-System Stability            | 18 days                                                                    |            |                      |

<sup>a</sup> A vertical bar in the page margin indicates technical content that differs from the previous version.

## Intended Use

The ADVIA Centaur® Testosterone II (TSTII) assay is for *in vitro* diagnostic use in the quantitative determination of total testosterone (bound and unbound) in human serum and plasma using the ADVIA Centaur® XP and ADVIA Centaur® XPT systems. For professional use.

Measurements of testosterone are used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females, hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries, and adrenogenital syndromes.

## Summary and Explanation

Testosterone (4 androsten 17 $\beta$ -ol-3-one) is a C<sub>19</sub> steroid hormone with a molecular weight of 288.4 daltons. Testosterone is the major androgen in males and is controlled by luteinizing hormone (LH). LH is released from the anterior pituitary exerting the primary control on testosterone production and acting directly on the Leydig cells in the testes. Testosterone stimulates adult maturation of external genitalia and secondary sex organs, and the growth of beard, axillary, and pubic hair.

In addition, testosterone has anabolic effects leading to increased linear growth, nitrogen retention, and muscular development. Clinical evaluation of serum testosterone, along with serum LH, assists in evaluation of hypogonadal males. Major causes of lowered testosterone in males include hypogonadotropic hypogonadism, testicular failure, hyperprolactinemia, hypopituitarism, some types of liver and kidney diseases, and critical illness.<sup>1</sup>

Testosterone levels are much lower in females compared to males. The major sources of testosterone in females are the ovaries, the adrenal glands, and the peripheral conversion of precursors, specifically the conversion of androstenedione to testosterone. In females, the normal levels of androgens may provide substrate for estrogen production. Increased serum testosterone levels in females may be indicative of polycystic ovary syndrome<sup>2</sup> and adrenal hyperplasia, among other conditions. The clinical manifestations of excess testosterone include infertility, hirsutism, amenorrhea, and obesity.<sup>3,4</sup>

Testosterone strongly binds to plasma proteins such as sex hormone-binding globulin (SHBG) or testosterone-estradiol-binding globulin (TeBG). Testosterone also binds with low affinity to cortisol-binding globulins (CBG) and albumin. Less than 2.5% of testosterone circulates unbound to plasma proteins.<sup>4</sup>

## Principles of the Procedure

The ADVIA Centaur TSTII assay is a competitive immunoassay using direct chemiluminescent technology. Testosterone in the sample competes with acridinium ester-labeled hapten in the Lite Reagent for binding with the anti-testosterone sheep monoclonal antibody in the ADVIA Centaur TSTII Releasing Agent and streptavidin magnetic latex particles in the Solid Phase Reagent. The ADVIA Centaur TSTII assay uses ADVIA Centaur TSTII Releasing Agent to release bound testosterone from the endogenous binding proteins in the sample.

## Reagents

| Reagent                                                                                                                                 | Description                                                                                                                                                                         | Storage | Reagent Stability                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------------------------------------------------------------------------------------------------------------------------------|
| ADVIA Centaur TSTII ReadyPack® primary reagent pack; Lite Reagent                                                                       | 10.0 mL/reagent pack<br>Acridinium ester-labeled hapten (36 µg/mL) in buffered saline with preservatives                                                                            | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 18 days                                  |
| ADVIA Centaur TSTII ReadyPack primary reagent pack; Solid Phase Reagent                                                                 | 17.0 mL/reagent pack<br>Streptavidin-coated latex particles (0.33 g/L) in buffered saline with preservatives                                                                        | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 18 days                                  |
| ADVIA Centaur TSTII ReadyPack ancillary reagent pack; Releasing Agent <span style="border: 1px solid black; padding: 0 2px;">REL</span> | 10.0 mL/reagent pack<br>Steroid releasing agent (0.4 µg/mL) and biotinylated sheep monoclonal anti-testosterone antibody (27 µg/L) in buffered saline and preservatives             | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 18 days                                  |
| ADVIA Centaur TSTII Calibrator                                                                                                          | 2.0 mL/vial<br>After reconstitution, low or high levels of USP-grade testosterone spiked in charcoal-stripped, defibrinated human plasma with sodium azide (0.1%) and preservatives | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>Reconstituted:</b> 14 days<br><b>On-system:</b> 6 hours |

| Reagent                                                                                                  | Description                                                                         | Storage | Reagent Stability                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| ADVIA Centaur ReadyPack ancillary reagent pack;<br>Probe Wash 1 <sup>a</sup> <b>APW</b> <b>1</b>         | 25.0 mL/reagent pack<br>0.4 N sodium hydroxide                                      | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 14 consecutive days after accessing the ancillary reagent pack |
| ADVIA Centaur ReadyPack primary reagent pack;<br>Probe Wash 4 <sup>a</sup> <b>PW</b> <b>4</b>            | 25.0 mL/reagent pack<br>0.25 N sodium hydroxide                                     | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 1 month                                                        |
| ADVIA Centaur ReadyPack ancillary reagent pack;<br>Multi-Diluent 3 <sup>b</sup><br><b>M-DIL</b> <b>3</b> | 5.0 mL/reagent pack<br>Human plasma with sodium azide (0.1%)                        | 2–8°C   | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 28 consecutive days after accessing the ancillary reagent pack |
| ADVIA Centaur Wash 1 <sup>a</sup> <b>WASH</b> <b>1</b>                                                   | 1500 mL/pack<br>Phosphate-buffered saline with sodium azide (< 0.1%) and surfactant | 2–25°C  | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 1 month                                                        |
| ADVIA Centaur Wash 1 <sup>a</sup> <b>WASH</b> <b>1</b>                                                   | 2500 mL/pack<br>Phosphate-buffered saline with sodium azide (< 0.1%) and surfactant | 2–25°C  | <b>Unopened:</b> Stable until the expiration date on the product<br><b>On-system:</b> 1 month                                                        |

<sup>a</sup> See *Materials Required but Not Provided*<sup>b</sup> See *Optional Materials*

## Warnings and Precautions

Safety data sheets (SDS) available on [siemens-healthineers.com](https://www.siemens-healthineers.com).



### Warning! Potential Biohazard

Contains human source material.

No known test method can ensure that products derived from human source materials will not transmit infection. These materials should be handled using good laboratory practices and universal precautions.<sup>5–7</sup>

### CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

**H412**  
**P273, P501**

Harmful to aquatic life with long lasting effects.  
Avoid release to the environment. Dispose of contents and container in accordance with all local, regional, and national regulations.  
**Contains:** sodium azide; ADVIA Centaur TSTII Calibrator



|                          |                                                                                                                                                                                                                                                                                                                                                             |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>H290</b>              | <b>Warning!</b>                                                                                                                                                                                                                                                                                                                                             |
| <b>H319</b>              | May be corrosive to metals.                                                                                                                                                                                                                                                                                                                                 |
| <b>H315</b>              | Causes serious eye irritation.                                                                                                                                                                                                                                                                                                                              |
| <b>P234, P264, P280,</b> | Causes skin irritation.                                                                                                                                                                                                                                                                                                                                     |
| <b>P337+P313, P390,</b>  | Keep only in original container. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. If eye irritation persists: Get medical advice/attention. Absorb spillage to prevent material damage. Dispose of contents and container in accordance with all local, regional, and national regulations. |
| <b>P501</b>              | <b>Contains:</b> sodium hydroxide (ADVIA Centaur Ancillary Probe Wash 1, ADVIA Centaur Probe Wash 4)                                                                                                                                                                                                                                                        |

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

For professional use.

**Caution:** Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

For *in vitro* diagnostic use.

## Preparing Reagents

All ADVIA Centaur TSTII ReadyPack reagents are liquid and ready to use. Remove all of the reagents from the refrigerator, and mix all primary reagent packs by hand. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended before loading them onto the system. For detailed information about preparing the reagents for use, refer to the system operating instructions.

**Note** The ADVIA Centaur TSTII Releasing Agent provided in this kit is matched to the Solid Phase and Lite Reagent. Do not mix ADVIA Centaur TSTII Releasing Agent lots with different lots of Solid Phase and Lite Reagent.

**Note** Do not use reagents beyond the expiration date.

## Storing and Stability

Store the reagents upright at 2–8°C.

Protect reagent packs from all heat and light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from heat and light sources.

All reagents are stable at 2–8°C until the expiration date on the packaging.

## Specimen Collection and Handling

Human serum and plasma (lithium heparin, sodium heparin, and dipotassium EDTA) are the recommended sample types for this assay.

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI).<sup>8</sup>

## Collecting the Specimen

- Collect all blood samples observing universal precautions for venipuncture.<sup>9</sup>
- Allow blood specimens to clot completely before centrifugation.<sup>8</sup>
- Do not use samples that have been stored at room temperature for longer than 8 hours prior to centrifugation.
- Tightly cap and refrigerate separated specimens at 2–8°C if the assay is not completed within 48 hours.
- Follow the instructions provided with your specimen collection device for use and processing.<sup>10</sup>

- Keep tubes stoppered and upright at all times.
- Samples with conjugated bilirubin concentrations > 15 mg/dL will cause erroneous results. Samples with unconjugated bilirubin concentrations > 20 mg/dL will cause erroneous results.

### Storing the Specimen

- Separated specimens are stable for 48 hours at room temperature, and for 7 days at 2–8°C. To store samples beyond these time periods, freeze samples at or below -20°C.<sup>11,12</sup>
- Freeze samples up to 3 times and mix thoroughly after thawing.
- Thawed frozen specimens that are turbid must be clarified by centrifugation prior to testing.
- Do not use separated specimens that have been stored at room temperature for longer than 48 hours.
- Tightly cap and refrigerate separated specimens at 2–8°C if the assay is not completed within 48 hours.

The purpose of handling and storage information is to provide guidance to users. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

### Transporting the Specimen

Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents.

## Procedure

### Materials Provided

The following materials are provided:

| REF      | Contents                                                                                                                                                                                                                                                                                                                                                                                                                                            | Number of Tests |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 10696862 | 1 ReadyPack primary reagent pack that contains ADVIA Centaur TSTII Lite Reagent, Solid Phase<br>1 ReadyPack ancillary reagent pack that contains ADVIA Centaur TSTII Releasing Agent <b>REL</b><br>1 vial of lyophilized ADVIA Centaur TSTII low calibrator <b>CAL L</b><br>1 vial of lyophilized ADVIA Centaur TSTII high calibrator <b>CAL H</b><br>ADVIA Centaur TSTII Master Curve Card<br>ADVIA Centaur TSTII Calibrator Assigned Value Card   | 100             |
| 10696863 | 5 ReadyPack primary reagent packs that contain ADVIA Centaur TSTII Lite Reagent, Solid Phase<br>5 ReadyPack ancillary reagent packs that contain ADVIA Centaur TSTII Releasing Agent <b>REL</b><br>2 vials of lyophilized ADVIA Centaur TSTII low calibrator <b>CAL L</b><br>2 vials of lyophilized ADVIA Centaur TSTII high calibrator <b>CAL H</b><br>ADVIA Centaur TSTII Master Curve Card<br>ADVIA Centaur TSTII Calibrator Assigned Value Card | 500             |

## Materials Required but Not Provided

The following materials are required to perform this assay, but are not provided:

| Item                         | Description                                                                                                   |
|------------------------------|---------------------------------------------------------------------------------------------------------------|
| <b>REF</b> 03395373          | ADVIA Centaur Ancillary Probe Wash 1 <b>APW</b> 1 2 ReadyPack ancillary reagent packs that contain 25 mL/pack |
| <b>REF</b> 04750940          | ADVIA Centaur Probe Wash 4 <b>PW</b> 4 1 ReadyPack primary reagent pack that contains 25 mL/pack              |
| <b>REF</b> 01137199 (112351) | ADVIA Centaur Wash 1 <b>WASH</b> 1 2 x 1500 mL/pack                                                           |
| <b>REF</b> 03773025          | ADVIA Centaur Wash 1 <sup>a</sup> <b>WASH</b> 1 2 x 2500 mL/pack                                              |

<sup>a</sup> for use with systems with 2500 mL capacity

## Optional Materials

The following materials may be used to perform this assay, but are not provided:

| Item                | Description                                                                                             |
|---------------------|---------------------------------------------------------------------------------------------------------|
| <b>REF</b> 05389133 | ADVIA Centaur Multi-Diluent 3 <b>M-DIL</b> 3 2 ReadyPack ancillary reagent packs that contain 5 mL/pack |
| <b>REF</b> 10492623 | ADVIA Centaur TSTII Master Curve Material <b>MCM</b>                                                    |

## Assay Procedure

For detailed instructions on performing the procedure, refer to the system operating instructions.

The system automatically performs the following actions:

1. Dispenses 20 µL of sample and 90 µL of Releasing Agent into a cuvette.
2. Incubates for 10.25 minutes at 37°C.
3. Dispenses 50 µL of Lite Reagent and 150 µL of Solid Phase and incubates for 3.75 minutes at 37°C.
4. Separates, aspirates, and washes the cuvettes with Wash 1.
5. Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
6. Reports results according to the selected option, as described in the system operating instructions.

An inverse relationship exists between the amount of testosterone present in the sample and the amount of relative light units (RLUs) detected by the system.

## Defining a Ratio Test for the Free Androgen Index (FAI)

The free androgen index (FAI) can be used to estimate the physiologically active testosterone.

The FAI is the ratio of total testosterone divided by SHBG (both expressed in the same units–nmol/L) and multiplied by 100 to yield numerical results comparable to the free testosterone concentration.<sup>13</sup>

$$\text{FAI (\%)} = \frac{\text{ADVIA Centaur TSTII (nmol/L)}}{\text{ADVIA Centaur SHBG (nmol/L)}} \times 100$$

**Note** This calculation can be performed on the ADVIA Centaur system or on the laboratory information system (LIS).

To perform the calculation on the ADVIA Centaur system, you must define a ratio test.

1. Assign a name to the ratio test (for example, FAI). You may use any name, however be sure to use the same name for the Ratio test name and the LIS Code.
2. In the Units field, enter %.
3. In the Decimal field, enter 2.
4. Follow the steps below for the appropriate system type.

#### **ADVIA Centaur XP**

On the ADVIA Centaur XP system the default ratio test calculation is displayed:

$$\frac{a - b}{c} \times d =$$

Define the following four variables of the ratio test calculation:

- Variable a: Select **Tests**. Then select TSTII in the field below.
- Variable b: Select **Variable**. Then enter 0 (zero) in the field below.
- Variable c: Select **Tests**. Then select SHBG in the field below.
- Variable d: Select **Variable**. Then enter 100 in the field below.

#### **ADVIA Centaur XPT**

On the ADVIA Centaur XPT system, define the formula in the Equation field so that the following appears: **(TSTII / SHBG) \* 100**

5. Click **Save**.

For more information about entering the ratio test definition for the FAI, refer to the system operating instructions.

## **Preparing the System**

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions.

Load the ReadyPack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents.

Load the ADVIA Centaur TSTII Releasing Agent reagent pack in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions.

If automatic dilution of a sample is required, load ADVIA Centaur Multi-Diluent 3 in the ancillary reagent entry.

## **Preparing the Samples**

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to the system operating instructions.

**Note** The sample volume required to perform onboard dilution differs from the sample volume required to perform a single determination. For detailed information, refer to *Dilutions*.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

## On-System Stability

The ADVIA Centaur TSTII assay reagents are stable unopened until the expiration date on the carton or onboard the system for 18 days.

## Defining Master Curve Values

The ADVIA Centaur TSTII assay requires that you define Master Curve values when using a new reagent lot number. For each new lot number of ReadyPack reagent, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve Card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions.

## Performing Calibration

For calibration of the ADVIA Centaur TSTII assay, use the ADVIA Centaur TSTII Calibrator.

**Note** The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

Each calibrator is packaged with a lot-specific Calibrator Assigned Value Card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. The Calibrator Assigned Value Card provided in this kit is matched to the ReadyPack primary reagent pack. For detailed information about entering calibrator values, refer to the system operating instructions.

## Preparing the Calibrators

Prepare calibrators using the following steps:

1. Add 2.0 mL of reagent water into each calibrator vial using a volumetric or precision pipet.

**Note** For information about reagent water, refer to the system operating instructions.

2. Let the calibrators stand for 30 minutes at room temperature (18–30°C) to allow the lyophilized material to dissolve.
3. Gently swirl and invert the vials until homogeneous.

## Calibration Procedure

For detailed information about processing calibrators, refer to the system operating instructions.

Perform the calibration procedure using the following steps:

1. Ensure that the appropriate master curve values are entered on the system. Refer to *Defining Master Curve Values*.
2. Enter the calibrator assigned values found on the ADVIA Centaur TSTII Calibrator Assigned Value Card into the system.
3. Ensure that the required reagents are loaded for the assay.
4. Schedule the ADVIA Centaur TSTII Calibrators to the workload.
5. Label 2 sample cups with ADVIA Centaur TSTII Calibrator barcode labels: 1 cup for the low calibrator and 1 cup for the high calibrator. Place the barcode label on the sample cup with the readable characters oriented vertically.

**Note** Calibrator barcode labels are lot-number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

6. Gently mix the low and high calibrators and dispense at least 350 µL of each calibrator into the appropriate sample cups. Avoid bubbles.
7. Load the calibrator sample cups in a sample rack.

8. Place the rack in the sample entry queue.
9. Start the entry queue, if required.

**Note** Dispose of any calibrator that remains in the sample cups after 6 hours. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

## Calibration Frequency

Calibrate the assay at the end of the 28-day calibration interval.

Additionally, the ADVIA Centaur TSTII assay requires a two-point calibration:

- When changing lot numbers of primary reagent packs.
- When replacing system components.
- When quality control results are repeatedly out of range.

## Performing Quality Control

To monitor system performance and chart trends, as a minimum requirement, two levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

Siemens Healthcare Diagnostics recommends the use of commercially available quality control materials with at least 2 levels (low and high). For assistance in identifying a quality control material, refer to *ADVIA Centaur Quality Control Material Supplement* available on [siemens-healthineers.com](http://siemens-healthineers.com).

Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

In addition, perform quality control:

- Following a valid calibration
- With use of a new lot of reagent
- When troubleshooting test results that do not match clinical conditions or symptoms

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system online help.

## Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

Perform corrective actions in accordance with your established laboratory protocol.

## Results

### Calculation of Results

For detailed information about how the system calculates results, refer to the system operating instructions.

The system reports serum testosterone results in ng/dL (common units) or nmol/L (SI units), depending on the units defined when setting up the assay.

The conversion formula is 1 ng/dL = 0.0347 nmol/L.

### Dilutions

The sample volume required to perform onboard dilution differs from the sample volume required to perform a single determination. Refer to the following information for the sample volume required to perform onboard dilutions:

| Dilution | Sample Volume (µL) |
|----------|--------------------|
| 1:2      | 70                 |

The following information pertains to dilutions:

- Dilute and retest serum samples with testosterone levels > 1500.00 ng/dL (> 52.05 nmol/L) to obtain accurate results.
- Patient samples can be automatically diluted by the system. Use ADVIA Centaur Multi-Diluent 3 to dilute patient samples. If a dilution factor is entered when scheduling the test, the system automatically calculates the result. For detailed information about dilutions, refer to the system operating instructions.
- For dilutions, ensure that ADVIA Centaur Multi-Diluent 3 is loaded on the system. Set the dilution factor to 2 and the dilution point to 1500.00 ng/dL (52.05 nmol/L).

### Interpretation of Results

Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

### Limitations

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.<sup>14</sup> Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- Samples with conjugated bilirubin concentrations > 15 mg/dL will cause erroneous results. Samples with unconjugated bilirubin concentrations > 20 mg/dL will cause erroneous results.
- A strong interaction with Nandrolone decanoate, 11β-hydroxytestosterone, and 11-keto-testosterone was found. Do not use samples from patients receiving these compounds.

With the advent of new steroid-based medications (analogues) with similar chemical structures to testosterone, there is the possibility of cross-reactivity and falsely elevated results. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings. If the testosterone results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

## Expected Values

Reference intervals for the ADVIA Centaur TSTII assay, ADVIA Centaur SHBG assay, and the Free Androgen Index (FAI) were obtained using samples from apparently healthy, normal adult females and males. The testosterone, SHBG, and FAI results provided in the tables below were obtained using results from the ADVIA Centaur TSTII and ADVIA Centaur SHBG assays.

Central 90% reference intervals were established in accordance with the CLSI guideline EP28-A3c<sup>15</sup> using a non-parametric approach for sample sizes of at least 120.

**Note** The FAI was calculated using the following equation:

$$\text{FAI (\%)} = \frac{\text{ADVIA Centaur TSTII (nmol/L)}}{\text{ADVIA Centaur SHBG (nmol/L)}} \times 100$$

### Testosterone - Adult males and females by age

| ADVIA Centaur TSTII<br>(years) | n   | Median<br>(ng/dL) | Central 90th<br>Reference Interval |                 | Median<br>(nmol/L) | Central 90th<br>Reference Interval |                  |
|--------------------------------|-----|-------------------|------------------------------------|-----------------|--------------------|------------------------------------|------------------|
|                                |     |                   | 5th<br>(ng/dL)                     | 95th<br>(ng/dL) |                    | 5th<br>(nmol/L)                    | 95th<br>(nmol/L) |
| Adult males < 50               | 250 | 409.72            | 197.44                             | 669.58          | 14.22              | 6.85                               | 23.23            |
| Adult males ≥ 50               | 135 | 377.46            | 187.72                             | 684.19          | 13.10              | 6.51                               | 23.74            |
| Adult females < 50             | 224 | 18.01             | 8.38                               | 35.01           | 0.62               | 0.29                               | 1.21             |
| Adult females ≥ 50             | 151 | 14.18             | < 7.00                             | 35.92           | 0.49               | < 0.24                             | 1.25             |

### SHBG - Adult males and females by age

| ADVIA Centaur SHBG<br>(years) | n   | Median<br>(µg/mL) | Central 90th<br>Reference Interval |                 | Median<br>(nmol/L) | Central 90th<br>Reference Interval |                  |
|-------------------------------|-----|-------------------|------------------------------------|-----------------|--------------------|------------------------------------|------------------|
|                               |     |                   | 5th<br>(µg/mL)                     | 95th<br>(µg/mL) |                    | 5th<br>(nmol/L)                    | 95th<br>(nmol/L) |
| Adult males < 50              | 250 | 2.26              | 1.10                               | 5.18            | 23.80              | 11.54                              | 54.49            |
| Adult males ≥ 50              | 135 | 3.51              | 1.65                               | 6.79            | 36.91              | 17.33                              | 71.50            |
| Adult females < 50            | 224 | 4.44              | 1.68                               | 13.13           | 46.72              | 17.69                              | 138.26           |
| Adult females ≥ 50            | 151 | 4.55              | 2.25                               | 10.51           | 47.86              | 23.65                              | 110.61           |

### FAI (%) - Adult males and females by age

| FAI<br>(years)     | n   | Median<br>(%) | Central 90th<br>Reference Interval |             |
|--------------------|-----|---------------|------------------------------------|-------------|
|                    |     |               | 5th<br>(%)                         | 95th<br>(%) |
| Adult males < 50   | 250 | 55.86         | 26.18                              | 107.07      |
| Adult males ≥ 50   | 135 | 36.01         | 17.38                              | 60.86       |
| Adult females < 50 | 224 | 1.37          | 0.33                               | 4.37        |
| Adult females ≥ 50 | 151 | 1.05          | 0.31                               | 2.53        |

Central 90% reference intervals characterized by age and Tanner stage were established for a pediatric population (children and adolescents) in accordance with the CLSI guideline EP28-A3c<sup>15</sup> using a non-parametric approach for sample sizes of at least 120. For populations with a sample size of 40–119, the central 90% reference interval was calculated using an approach to accommodate the smaller sample size.<sup>16</sup> For sample sizes of < 40, the reference interval is defined by the 5th and 95th percentiles.

Samples were collected prospectively from apparently healthy pediatric subjects (good endocrinological health) using predefined inclusion criteria.

#### Testosterone - Pediatric males and females by age

| ADVIA Centaur TSTII<br>(years) | n   | Median<br>(ng/dL) | Central 90th<br>Reference Interval |                 | Median<br>(nmol/L) | Central 90th<br>Reference Interval |                  |
|--------------------------------|-----|-------------------|------------------------------------|-----------------|--------------------|------------------------------------|------------------|
|                                |     |                   | 5th<br>(ng/dL)                     | 95th<br>(ng/dL) |                    | 5th<br>(nmol/L)                    | 95th<br>(nmol/L) |
| Pediatric males                |     |                   |                                    |                 |                    |                                    |                  |
| Age 2–10                       | 147 | < 7.00            | < 7.00                             | 10.50           | < 0.24             | < 0.24                             | 0.36             |
| Age 11                         | 34  | 10.73             | < 7.00                             | 478.50          | 0.37               | < 0.24                             | 16.60            |
| Age 12                         | 35  | 132.47            | < 7.00                             | 487.97          | 4.60               | < 0.24                             | 16.93            |
| Age 13                         | 34  | 199.02            | 8.28                               | 549.79          | 6.91               | 0.29                               | 19.08            |
| Age 14                         | 34  | 228.39            | 8.91                               | 535.34          | 7.93               | 0.31                               | 18.58            |
| Age 15                         | 27  | 327.89            | 65.96                              | 756.50          | 11.38              | 2.29                               | 26.25            |
| Age 16–21                      | 149 | 453.86            | 228.16                             | 710.74          | 15.75              | 7.92                               | 24.66            |
| Pediatric females              |     |                   |                                    |                 |                    |                                    |                  |
| Age 2–10                       | 159 | < 7.00            | < 7.00                             | 11.86           | < 0.24             | < 0.24                             | 0.41             |
| Age 11–15                      | 174 | 12.95             | < 7.00                             | 27.57           | 0.45               | < 0.24                             | 0.96             |
| Age 16–21                      | 145 | 19.81             | 11.78                              | 43.34           | 0.69               | 0.41                               | 1.50             |

#### SHBG - Pediatric males and females by age

| ADVIA Centaur SHBG<br>(years) | n   | Median<br>(µg/mL) | Central 90th<br>Reference Interval |                 | Median<br>(nmol/L) | Central 90th<br>Reference Interval |                  |
|-------------------------------|-----|-------------------|------------------------------------|-----------------|--------------------|------------------------------------|------------------|
|                               |     |                   | 5th<br>(µg/mL)                     | 95th<br>(µg/mL) |                    | 5th<br>(nmol/L)                    | 95th<br>(nmol/L) |
| Pediatric males               |     |                   |                                    |                 |                    |                                    |                  |
| Age 2–10                      | 147 | 8.90              | 3.29                               | 15.42           | 93.68              | 34.64                              | 162.29           |
| Age 11                        | 34  | 5.62              | 1.68                               | 10.90           | 59.18              | 17.66                              | 114.73           |
| Age 12                        | 35  | 4.81              | 1.45                               | 11.06           | 50.60              | 15.24                              | 116.39           |
| Age 13                        | 34  | 3.02              | 1.39                               | 10.37           | 31.83              | 14.67                              | 109.13           |
| Age 14                        | 34  | 3.00              | 1.24                               | 7.66            | 31.57              | 13.07                              | 80.64            |
| Age 15                        | 27  | 2.51              | 1.12                               | 3.84            | 26.45              | 11.84                              | 40.47            |
| Age 16–21                     | 149 | 2.38              | 1.05                               | 4.73            | 25.05              | 11.08                              | 49.80            |

**SHBG - Pediatric males and females by age**

|                               |     |                   | Central 90th<br>Reference Interval |                 | Central 90th<br>Reference Interval |                 |                  |
|-------------------------------|-----|-------------------|------------------------------------|-----------------|------------------------------------|-----------------|------------------|
| ADVIA Centaur SHBG<br>(years) | n   | Median<br>(µg/mL) | 5th<br>(µg/mL)                     | 95th<br>(µg/mL) | Median<br>(nmol/L)                 | 5th<br>(nmol/L) | 95th<br>(nmol/L) |
| Pediatric females             |     |                   |                                    |                 |                                    |                 |                  |
| Age 2–10                      | 160 | 7.18              | 2.76                               | 15.05           | 75.55                              | 29.07           | 158.46           |
| Age 11–15                     | 175 | 4.53              | 1.48                               | 9.67            | 47.72                              | 15.62           | 101.74           |
| Age 16–21                     | 145 | 4.45              | 1.84                               | 15.37           | 46.81                              | 19.36           | 161.78           |

**FAI (%) - Pediatric males and females by age**

| FAI<br>(years)    | n   | Median<br>(%) | Central 90th<br>Reference Interval |             |
|-------------------|-----|---------------|------------------------------------|-------------|
|                   |     |               | 5th<br>(%)                         | 95th<br>(%) |
| Pediatric males   |     |               |                                    |             |
| Age 2–10          | 147 | 0.07          | < 0.07                             | 1.09        |
| Age 11            | 34  | 0.59          | 0.07                               | 56.77       |
| Age 12            | 35  | 7.90          | 0.20                               | 60.95       |
| Age 13            | 34  | 23.33         | 0.30                               | 71.07       |
| Age 14            | 34  | 28.36         | 0.53                               | 71.17       |
| Age 15            | 27  | 46.41         | 8.63                               | 80.53       |
| Age 16–21         | 149 | 62.30         | 33.19                              | 109.15      |
| Pediatric females |     |               |                                    |             |
| Age 2–10          | 159 | 0.14          | < 0.07                             | 0.91        |
| Age 11–15         | 174 | 0.98          | 0.26                               | 3.86        |
| Age 16–21         | 145 | 1.50          | 0.42                               | 5.29        |

Samples were also clinically characterized according to Tanner Stage:

**Testosterone - Pediatric males and females by Tanner Stage**

|                               |     |                   | Central 90th<br>Reference Interval |                 | Central 90th<br>Reference Interval |                 |                  |
|-------------------------------|-----|-------------------|------------------------------------|-----------------|------------------------------------|-----------------|------------------|
| ADVIA Centaur TSTII           | n   | Median<br>(ng/dL) | 5th<br>(ng/dL)                     | 95th<br>(ng/dL) | Median<br>(nmol/L)                 | 5th<br>(nmol/L) | 95th<br>(nmol/L) |
| Pediatric males <sup>17</sup> |     |                   |                                    |                 |                                    |                 |                  |
| Tanner Stage 1                | 101 | < 7.00            | < 7.00                             | 13.06           | < 0.24                             | < 0.24          | 0.45             |
| Tanner Stage 2                | 78  | < 7.00            | < 7.00                             | 79.13           | < 0.24                             | < 0.24          | 2.75             |
| Tanner Stage 3                | 64  | 59.67             | < 7.00                             | 499.18          | 2.07                               | < 0.24          | 17.32            |
| Tanner Stage 4                | 88  | 376.84            | 79.10                              | 747.17          | 13.08                              | 2.74            | 25.93            |
| Tanner Stage 5                | 129 | 451.17            | 224.83                             | 669.65          | 15.66                              | 7.80            | 23.24            |

## Testosterone - Pediatric males and females by Tanner Stage

|                                 |     |                   | Central 90th<br>Reference Interval |                 | Central 90th<br>Reference Interval |                 |                  |
|---------------------------------|-----|-------------------|------------------------------------|-----------------|------------------------------------|-----------------|------------------|
| ADVIA Centaur TSTII             | n   | Median<br>(ng/dL) | 5th<br>(ng/dL)                     | 95th<br>(ng/dL) | Median<br>(nmol/L)                 | 5th<br>(nmol/L) | 95th<br>(nmol/L) |
| Pediatric females <sup>18</sup> |     |                   |                                    |                 |                                    |                 |                  |
| Tanner Stage 1                  | 138 | < 7.00            | < 7.00                             | 10.06           | < 0.24                             | < 0.24          | 0.35             |
| Tanner Stage 2                  | 60  | 8.17              | < 7.00                             | 30.11           | 0.28                               | < 0.24          | 1.04             |
| Tanner Stage 3                  | 49  | 12.98             | < 7.00                             | 30.49           | 0.45                               | < 0.24          | 1.06             |
| Tanner Stage 4                  | 98  | 17.37             | < 7.00                             | 35.19           | 0.60                               | < 0.24          | 1.22             |
| Tanner Stage 5                  | 133 | 19.76             | 11.80                              | 39.30           | 0.69                               | 0.41            | 1.36             |

## SHBG - Pediatric males and females by Tanner Stage

| ADVIA Centaur SHBG              | n   | Median<br>(µg/mL) | Central 90th<br>Reference Interval |                 | Central 90th<br>Reference Interval |                 |                  |
|---------------------------------|-----|-------------------|------------------------------------|-----------------|------------------------------------|-----------------|------------------|
|                                 |     |                   | 5th<br>(µg/mL)                     | 95th<br>(µg/mL) | Median<br>(nmol/L)                 | 5th<br>(nmol/L) | 95th<br>(nmol/L) |
| Pediatric males <sup>17</sup>   |     |                   |                                    |                 |                                    |                 |                  |
| Tanner Stage 1                  | 101 | 9.26              | 4.03                               | 15.35           | 97.44                              | 42.43           | 161.54           |
| Tanner Stage 2                  | 78  | 7.16              | 2.53                               | 13.91           | 75.38                              | 26.61           | 146.40           |
| Tanner Stage 3                  | 64  | 4.39              | 1.32                               | 10.29           | 46.23                              | 13.91           | 108.35           |
| Tanner Stage 4                  | 88  | 2.73              | 1.16                               | 6.12            | 28.78                              | 12.18           | 64.42            |
| Tanner Stage 5                  | 129 | 2.38              | 1.21                               | 4.65            | 25.05                              | 12.77           | 48.93            |
| Pediatric females <sup>18</sup> |     |                   |                                    |                 |                                    |                 |                  |
| Tanner Stage 1                  | 139 | 7.90              | 2.81                               | 15.28           | 83.20                              | 29.56           | 160.80           |
| Tanner Stage 2                  | 61  | 5.14              | 1.55                               | 10.35           | 54.12                              | 16.29           | 108.98           |
| Tanner Stage 3                  | 49  | 4.71              | 1.50                               | 12.91           | 49.59                              | 15.83           | 135.89           |
| Tanner Stage 4                  | 98  | 4.33              | 1.67                               | 9.47            | 45.58                              | 17.53           | 99.70            |
| Tanner Stage 5                  | 133 | 4.39              | 1.88                               | 13.40           | 46.21                              | 19.75           | 141.10           |

## FAI (%) - Pediatric males and females by Tanner stage

| FAI                           | n   | Median (%) | Central 90th Reference Interval |          |
|-------------------------------|-----|------------|---------------------------------|----------|
|                               |     |            | 5th (%)                         | 95th (%) |
| Pediatric males <sup>17</sup> |     |            |                                 |          |
| Tanner Stage 1                | 101 | 0.07       | < 0.07                          | 0.72     |
| Tanner Stage 2                | 78  | 0.19       | < 0.07                          | 6.78     |
| Tanner Stage 3                | 64  | 7.98       | < 0.07                          | 56.18    |
| Tanner Stage 4                | 88  | 47.74      | 8.13                            | 99.66    |
| Tanner Stage 5                | 129 | 61.22      | 30.87                           | 98.86    |

**FAI (%) - Pediatric males and females by Tanner stage**

| FAI                             | n   | Median (%) | Central 90th Reference Interval |          |
|---------------------------------|-----|------------|---------------------------------|----------|
|                                 |     |            | 5th (%)                         | 95th (%) |
| Pediatric females <sup>18</sup> |     |            |                                 |          |
| Tanner Stage 1                  | 138 | 0.10       | < 0.07                          | 0.81     |
| Tanner Stage 2                  | 60  | 0.57       | 0.09                            | 3.47     |
| Tanner Stage 3                  | 49  | 1.01       | 0.26                            | 3.75     |
| Tanner Stage 4                  | 98  | 1.17       | 0.40                            | 5.00     |
| Tanner Stage 5                  | 133 | 1.43       | 0.44                            | 5.29     |

The Clinical Practice Guideline from the Endocrine Society provided harmonized reference intervals for total testosterone in healthy, non-obese men (aged 19–39 years) for assays that demonstrate alignment to the CDC HoST Testosterone Reference Measurement Procedure.<sup>19</sup>

**Endocrine Society Harmonized 90th and Central 95th Reference Intervals - Men Aged 19–39**

| Reference Interval |                                  |
|--------------------|----------------------------------|
| Central 90th       | 303–852 ng/dL (10.5–29.5 nmol/L) |
| Central 95th       | 264–916 ng/dL (9.2–31.8 nmol/L)  |

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference ranges for the diagnostic evaluation of patient results. Consider these ranges as guidelines only.

## Performance Characteristics

### Measuring Interval

The ADVIA Centaur TSTII assay measures testosterone from concentrations of 7.00–1500.00 ng/dL (0.24–52.05 nmol/L). The low end of the assay range is defined by the limit of quantitation (LoQ).

### Specificity

The ADVIA Centaur TSTII assay shows high specificity for testosterone. The following compounds were tested with total testosterone concentrations targeted at 0 ng/dL and 300 ng/dL. Percent change is calculated as:

Percent cross-reactivity = (corrected assay value / amount of compound spiked) x 100

The following results were obtained:

| Cross-reactant                            | Cross-reactant Concentration (ng/mL) | Maximum Observed Cross-reactivity (%) |
|-------------------------------------------|--------------------------------------|---------------------------------------|
| 5-Androstene-3 $\beta$ , 17 $\beta$ -diol | 1000                                 | -0.167                                |
| Androstenedione                           | 100                                  | 1.397                                 |
| Androsterone                              | 1000                                 | -0.026                                |
| Canrenone                                 | 180                                  | 0.066                                 |
| Canrenone                                 | 500                                  | 0.005                                 |
| Canrenone                                 | 1000                                 | 0.008                                 |
| Corticosterone                            | 1000                                 | 0.014                                 |

| Cross-reactant                               | Cross-reactant Concentration (ng/mL) | Maximum Observed Cross-reactivity (%) |
|----------------------------------------------|--------------------------------------|---------------------------------------|
| Cortisol                                     | 1000                                 | 0.022                                 |
| Cyproterone                                  | 100                                  | 0.128                                 |
| Danazol                                      | 1000                                 | -0.121                                |
| 11-Deoxycortisol                             | 1000                                 | 0.002                                 |
| Dexamethasone                                | 1000                                 | 0.006                                 |
| DHEA                                         | 1000                                 | -0.042                                |
| DHEAs                                        | 50,000                               | 0.001                                 |
| 5 $\alpha$ -Dihydrotestosterone              | 100                                  | -1.410                                |
| 17 $\beta$ -Estradiol                        | 1000                                 | -0.267                                |
| Estrone                                      | 100                                  | -0.009                                |
| Ethisterone                                  | 1000                                 | 0.446                                 |
| 11 $\beta$ -Hydroxytestosterone <sup>a</sup> | 100                                  | 15.469                                |
| 11-Keto-testosterone <sup>a</sup>            | 1000                                 | 1.766                                 |
| Nandrolone decanoate <sup>a</sup>            | 1000                                 | NA <sup>b</sup>                       |
| Norgestrel                                   | 1000                                 | 0.145                                 |
| Oxymetholone                                 | 100                                  | -0.576                                |
| Prednisolone                                 | 1000                                 | 0.093                                 |
| Prednisone                                   | 1000                                 | 0.011                                 |
| Progesterone                                 | 1000                                 | 0.014                                 |
| Spironolactone                               | 80                                   | 0.105                                 |
| Spironolactone                               | 1000                                 | 0.011                                 |
| Testosterone propionate                      | 100                                  | 2.946                                 |
| 7 $\alpha$ -Thiomethyl spironolactone        | 400                                  | 0.001                                 |
| 7 $\alpha$ -Thiomethyl spironolactone        | 1000                                 | -0.002                                |

<sup>a</sup> Refer to *Limitations*.

<sup>b</sup> Cross-reactivity for this compound could not be calculated. This compound tested above the measuring interval.

## Detection Capability

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined as described in CLSI protocol EP17-A2.<sup>20</sup> The ADVIA Centaur TSTII assay has an LoB of 2.50 ng/dL (0.09 nmol/L), an LoD of 5.00 ng/dL (0.17 nmol/L), and an LoQ of 7.00 ng/dL (0.24 nmol/L).

The LoB is defined as the highest measurement result that is likely to be observed for a blank sample. The LoD is defined as the lowest concentration of testosterone that can be detected with 95% probability. The LoQ is defined as the lowest concentration of testosterone that can be detected at a total CV of 20%.

## Precision

The ADVIA Centaur TSTII assay is designed to meet the following repeatability and within-lab precision requirements:

| Testosterone Level |              | Design Requirements               |                                     |
|--------------------|--------------|-----------------------------------|-------------------------------------|
| (ng/dL)            | (nmol/L)     | Repeatability (Within-Run)<br>%CV | Within-Lab (Total Precision)<br>%CV |
| ≥ 15.00–20.00      | ≥ 0.52–0.69  | ≤ 18.0                            | ≤ 20%                               |
| > 20.00–1000.00    | > 0.69–34.70 | ≤ 8.0                             | ≤ 10%                               |
| > 1000.00          | > 34.70      | ≤ 11.0                            | ≤ 12%                               |

Precision was evaluated according to the CLSI protocol EP05-A3.<sup>21</sup> Nine samples were assayed twice a day in replicates of 2, for 20 days (n = 80 replicates per sample) using the ADVIA Centaur TSTII assay. The following results were obtained:

| Sample                    | n  | Mean    |          | Repeatability (Within-Run) |             |      | Within-Lab (Total Precision) |             |      |
|---------------------------|----|---------|----------|----------------------------|-------------|------|------------------------------|-------------|------|
|                           |    | (ng/dL) | (nmol/L) | SD (ng/dL)                 | SD (nmol/L) | %CV  | SD (ng/dL)                   | SD (nmol/L) | %CV  |
| Control 1                 | 80 | 257.38  | 8.93     | 9.06                       | 0.31        | 3.5  | 14.38                        | 0.50        | 5.6  |
| Control 2                 | 80 | 636.57  | 22.09    | 45.14                      | 1.57        | 7.1  | 53.12                        | 1.84        | 8.3  |
| Control 3                 | 80 | 1021.93 | 35.46    | 62.28                      | 2.16        | 6.1  | 81.02                        | 2.81        | 7.9  |
| Patient pool 1            | 80 | 20.92   | 0.73     | 1.3                        | 0.05        | 6.2  | 1.86                         | 0.06        | 8.9  |
| Patient pool 2            | 80 | 73.57   | 2.55     | 3.54                       | 0.12        | 4.8  | 5.24                         | 0.18        | 7.1  |
| Patient pool 3            | 80 | 312.81  | 10.85    | 13.4                       | 0.47        | 4.3  | 23.99                        | 0.83        | 7.7  |
| Patient pool 4            | 80 | 776.64  | 26.95    | 42.59                      | 1.48        | 5.5  | 58.26                        | 2.02        | 7.5  |
| Patient pool 5            | 80 | 1123.83 | 39.00    | 59.22                      | 2.06        | 5.3  | 109.57                       | 3.80        | 9.7  |
| Female patient serum pool | 80 | 15.86   | 0.55     | 1.84                       | 0.06        | 11.6 | 2.39                         | 0.08        | 15.1 |

Based on internal testing, the overall reproducibility is estimated to be ≤ 20% CV for samples tested and includes multiple reagent lots, instruments, days, and replicates. Performance of the assay at individual laboratories may vary.

## Method Comparison

The ADVIA Centaur TSTII assay is designed to have a correlation coefficient ≥ 0.95 when compared to an isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) testosterone method (Centers for Disease Control and Prevention Hormone Standardization Program (CDC HoSt) Testosterone Reference Measurement Procedure). Typical data obtained showed 0.98.

For 108 samples in the range of 7.27–1394.00 ng/dL (0.25–48.37 nmol/L), the relationship between the ADVIA Centaur TSTII assay (y) and the ID-LC-MS/MS (x) is described using Weighted Deming regression:<sup>22</sup>

ADVIA Centaur TSTII (y) = 0.97 (x) (ID-LC-MS/MS) - 0.22 ng/dL (0.01 nmol/L), r = 0.98\*

\* The correlation coefficient was obtained from ordinary least squares regression.

An additional method comparison study was performed against the Dimension Vista® Total Testosterone (TTST) assay with 124 serum samples (79 samples from adults and 45 samples from pediatric subjects) ranging from 9.00–984.00 ng/dL (0.31–34.12 nmol/L). An analysis was also performed with the 45 samples from pediatric subjects (31 females, ages 7 years to 18 years; and 14 males, ages 22 months to 18 years) ranging from 11.00–762.00 ng/dL (0.38–26.44 nmol/L). The analyses were performed using Weighted Deming regression. The regression equations from the analyses are:

#### Adult and pediatric specimens

ADVIA Centaur TSTII (y) = 1.01 (x) (Dimension Vista TTST) - 3.32 ng/dL (0.12 nmol/L) (r = 0.99)

#### Pediatric specimens

ADVIA Centaur TSTII (y) = 1.01 (x) (Dimension Vista TTST) - 4.74 ng/dL (0.16 nmol/L) (r = 1.00)

The correlation of the assay may vary depending on the study design, comparable method, and sample population. Assay results obtained at individual laboratories may vary from the data presented.

## Specimen Collection Comparison

The ADVIA Centaur TSTII assay was evaluated using different specimen matrices and tube collection types. A specimen collection study was performed using a minimum of 50 matched specimens drawn in different tube types including serum red top, serum gel barrier tube, plasma gel barrier tube, dipotassium EDTA, lithium heparin, and sodium heparin.

Testosterone values ranged from 8.50–1195.55 ng/dL (0.29–41.49 nmol/L). Linear regression analysis was performed using the following tube types:

- serum (x) vs. serum gel barrier tube (y<sub>1</sub>)
- serum (x) vs. plasma gel barrier tube (y<sub>2</sub>)
- serum (x) vs. dipotassium EDTA (y<sub>3</sub>)
- serum (x) vs. lithium heparin (y<sub>4</sub>)
- serum (x) vs. sodium heparin (y<sub>5</sub>)

No significant difference between tube types was observed. The following results were obtained:

| Tube Types                        | Slope | Intercept |          |       |
|-----------------------------------|-------|-----------|----------|-------|
|                                   |       | (ng/dL)   | (nmol/L) | r     |
| Serum vs. serum gel barrier tube  | 0.98  | 3.76      | 0.13     | 0.991 |
| Serum vs. plasma gel barrier tube | 0.94  | 3.56      | 0.12     | 0.993 |
| Serum vs. dipotassium EDTA        | 0.97  | -1.61     | -0.06    | 0.994 |
| Serum vs. lithium heparin         | 0.95  | 4.50      | 0.16     | 0.996 |
| Serum vs. sodium heparin          | 0.94  | 2.85      | 0.10     | 0.996 |

## Interferences

Potential interference in the ADVIA Centaur TSTII assay from hemoglobin, triglycerides, and bilirubin is designed to be  $\leq 10\%$ . Interfering substances at the levels indicated in the table below were tested as described in CLSI Document EP07-A2<sup>23</sup> using the ADVIA Centaur TSTII assay. Assay results obtained at individual laboratories may vary from the data presented.

| Specimens that are... | Demonstrate $\leq 10\%$ change in results up to... |
|-----------------------|----------------------------------------------------|
| Hemolyzed             | 500 mg/dL of hemoglobin                            |
| Lipemic               | 1000 mg/dL of triglycerides                        |
| Icteric               | 15 mg/dL of conjugated bilirubin                   |
| Icteric               | 20 mg/dL of unconjugated bilirubin                 |

Samples with conjugated bilirubin concentrations  $> 15$  mg/dL will cause erroneous results. Specimens that contain 20 mg/dL of conjugated bilirubin demonstrate a -14.4% change in results.

Samples with unconjugated bilirubin concentrations  $> 20$  mg/dL will cause erroneous results. Specimens that contain 30 mg/dL of unconjugated bilirubin demonstrate a 20.1% change in results.

| Specimens that contain... | Demonstrate $\leq 10\%$ change in results up to... |
|---------------------------|----------------------------------------------------|
| Biotin                    | 30 ng/mL of biotin                                 |
| Cholesterol               | 500 mg/dL of cholesterol                           |
| Rheumatoid Factor         | 200 IU/mL of rheumatoid sample                     |

Samples with biotin concentrations  $> 30$  ng/mL will cause erroneous results. Specimens that contain 40 ng/mL of biotin at a testosterone level of 30 ng/dL (1.04 nmol/L) demonstrate a 14.6% change in results.

An additional study was carried out to assess the effect of biotin interference up to 1500 ng/mL. The results are shown in the table below:

| Analyte Concentration (ng/dL) | Biotin Test Level (ng/mL) |    |     |     |     |          |        |
|-------------------------------|---------------------------|----|-----|-----|-----|----------|--------|
|                               | 30                        | 50 | 100 | 250 | 500 | 1200     | 1500   |
|                               | % Bias                    |    |     |     |     |          |        |
| 83.27                         | 4                         | 6  | 23  | 84  | 370 | $> MI^a$ | $> MI$ |
| 289.24                        | 2                         | 7  | 19  | 107 | 441 | $> MI$   | $> MI$ |

<sup>a</sup> MI = measuring interval

Specimens that contain biotin at a concentration of 30 ng/mL demonstrate a less than or equal to 10% change in results. Biotin concentrations greater than this may lead to falsely elevated results for patient samples.

The recommended adult daily dietary intake for biotin is 30  $\mu$ g/day. Over the counter dietary supplements promoted for use in hair, skin, and nail health may contain 5–100 mg of biotin, with recommendations to take multiple pills per day. Pharmacokinetic studies in healthy adults have shown that, in subjects ingesting 5 mg, 10 mg, and 20 mg of biotin, serum concentrations of biotin can reach up to 73 ng/mL, 141 ng/mL, and 355 ng/mL, respectively.<sup>24</sup> Subjects who take up to 300 mg of biotin per day may have plasma biotin levels as high as 1160 ng/mL.<sup>25</sup>

## Linearity

Linearity was evaluated using a sample that contained a high level of testosterone, which was mixed in various proportions with a sample at a low level of testosterone. The resulting sample mixtures (9 combinations) were assayed for testosterone.

The ADVIA Centaur TSTII assay is linear from 7.00–1500.00 ng/dL (0.24–52.05 nmol/L).

## Dilution Recovery

Five serum samples in the range of 1520.24–1715.58 ng/dL (52.75–59.53 nmol/L) of testosterone were diluted 1:2 with ADVIA Centaur Multi-Diluent 3, and assayed for recovery. The recoveries ranged from 93.9%–114.2%, with a mean of 106.6%.

| Sample | Dilution | Observed |          | Expected |          | Recovery (%) |
|--------|----------|----------|----------|----------|----------|--------------|
|        |          | (ng/dL)  | (nmol/L) | (ng/dL)  | (nmol/L) |              |
| 1      | 1:2      | 1581.78  | 54.89    | 1684.06  | 58.44    | 93.9         |
| 2      | 1:2      | 1757.54  | 60.99    | 1636.02  | 56.77    | 107.4        |
| 3      | 1:2      | 1730.52  | 60.05    | 1520.24  | 52.75    | 113.8        |
| 4      | 1:2      | 1825.78  | 63.35    | 1598.78  | 55.48    | 114.2        |
| 5      | 1:2      | 1782.64  | 61.86    | 1715.58  | 59.53    | 103.9        |
| Mean   |          |          |          |          |          | 106.6        |

## Standardization

The ADVIA Centaur TSTII assay is standardized using internal standards made from USP-grade testosterone, which are traceable to isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) (Centers for Disease Control and Prevention Hormone Standardization Program (CDC HoSt) Testosterone Reference Measurement Procedure). The ID-LC-MS/MS is traceable to the primary testosterone standard National Measurement Institute (NMI) M914.<sup>26</sup>

## Technical Assistance

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

For customer support, please contact your local technical support provider or distributor.

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## References

1. Spratt DI, Bigos ST, Beitins I, et al. Both hyper- and hypogonadotropic hypogonadism occur transiently in acute illness: bio- and immunoactive gonadotropins. *J Clin Endocrinol Metab.* 1992;(7)1562–1570.
2. Anttila L, Koskinen P, Erkkola R, et al. Serum testosterone, androstenedione, and luteinizing hormone levels after short-term medroxyprogesterone acetate treatment in women with polycystic ovarian disease. *Acta Obstet et Gynecol Scand.* 1994;73:634–636.
3. Wilke TJ, Utley DJ. Total testosterone, free-androgen index, calculated free testosterone and free testosterone by analog RIA compared in hirsute women and in otherwise-normal women with altered binding of sex-hormone-binding globulin. *Clin Chem.* 1978;33:8.
4. Kicklighter EJ, Norman RJ. The gonads. In: Kaplan LA, Pesce AJ, eds. *Clinical Chemistry: Theory, Analysis, Correlation.* 2nd ed. St. Louis: CV Mosby; 1989:657–662.

5. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
6. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
7. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI Document M29-A4.
8. Clinical and Laboratory Standards Institute. *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline—Fourth Edition*. CLSI Document GP44-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
9. Clinical and Laboratory Standards Institute. *Procedures for the Collection of Diagnostic of Blood Specimens by Venipuncture; Approved Guideline—Sixth Edition*. CLSI Document GP41-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
10. Clinical and Laboratory Standards Institute. *Tubes and Additives for Venous and Capillary Blood Specimen Collection: Approved Standard—Sixth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. CLSI document GP39-A6; 2010.
11. Kley HK, Schlaghecke R, Krüskemper HL. Stability of steroids in plasma over a 10-year period. *J. Clin Chem. and Clin Biochem.* 1985;23(12):875–878.
12. Stroud LR, Solomon C, Shenassa E, et al. Long-term stability of maternal prenatal steroid hormones from the National Collaborative Perinatal Project: still valid after all these years. *Psychoneuroendocrinology*. 2007 Feb;32(2):140-150.
13. Wilke TJ, Utley DJ. Total testosterone, free-androgen index, calculated free testosterone, and free testosterone by analog RIA compared in hirsute women and in otherwise-normal women with altered sex-hormone-binding globulin. *Clin Chem.* 1987;33:1372–1375.
14. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem.* 1988;34:27–33.
15. Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition*. CLSI Document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
16. Horn PS, Pesce AJ. *Reference Intervals. A User's Guide*. Washington, DC: AACC Press; 2005.
17. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Childh.* 1970;45:13–23.
18. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. *Arch Dis Childh.* 1969;44:291–303.
19. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2018 May 1;103(5):1715–1744.
20. Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition*. CLSI Document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
21. Clinical and Laboratory Standards Institute. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition*. CLSI Document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
22. Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition*. CLSI Document EP09-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

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23. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*. CLSI Document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
  24. Grimsey P, Frey N, Bendig G, et al. Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and *in vitro* immunoassay interference. *Int. J. Pharmacokinet.* 2017;2(4),247–256.
  25. Piketty ML, Prie D, Sedel F, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. *Clin Chem Lab Med.* 2017 May 1;55(6):817–825.
  26. Botelho JC, Shacklady C, Cooper HC, et al. Isotope-Dilution Liquid Chromatography - Tandem Mass Spectrometry Candidate Reference Method for Total Testosterone in Human Serum. *Clin Chem.* 2013;59:2;372–380.

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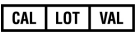
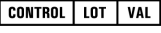
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US Pats 8,778,624; 9,575,062

## Definition of Symbols

The following symbols may appear on the product labeling:

| Symbol        | Symbol Title                                                       | Source               | Symbol  | Symbol Title                                        | Source               |
|---------------|--------------------------------------------------------------------|----------------------|---------|-----------------------------------------------------|----------------------|
|               | Manufacturer                                                       | 5.1.1 <sup>a</sup>   |         | Authorized representative in the European Community | 5.1.2 <sup>a</sup>   |
|               | Use-by date                                                        | 5.1.4 <sup>a</sup>   |         | Authorized representative in Switzerland            | Proprietary          |
|               | Catalog number                                                     | 5.1.6 <sup>a</sup>   |         | Batch code                                          | 5.1.5 <sup>a</sup>   |
|               | Consult Instructions for Use                                       | 5.4.3 <sup>a</sup>   |         | Contains sufficient for <n> tests                   | 5.5.5 <sup>a</sup>   |
|               | Internet URL address to access the electronic instructions for use | Proprietary          |         | Version of Instructions for Use                     | Proprietary          |
|               | <i>In vitro</i> diagnostic medical device                          | 5.5.1 <sup>a</sup>   |         | Revision                                            | Proprietary          |
| <b>RxOnly</b> | Prescription device (US only)                                      | FDA <sup>c</sup>     |         | Unique Device Identifier                            | 5.7.10 <sup>b</sup>  |
|               | CE Marking with Notified Body                                      | EU IVDR <sup>d</sup> |         | CE Marking                                          | EU IVDR <sup>d</sup> |
|               | Temperature limit                                                  | 5.3.7 <sup>a</sup>   |         | Keep away from sunlight                             | 5.3.2 <sup>a</sup>   |
|               | Upper limit of temperature                                         | 5.3.6 <sup>a</sup>   |         | Lower limit of temperature                          | 5.3.5 <sup>a</sup>   |
|               | Do not re-use                                                      | 5.4.2 <sup>a</sup>   |         | Do not freeze                                       | Proprietary          |
|               | Recycle                                                            | 1135 <sup>e</sup>    |         | This way up                                         | 0623 <sup>e</sup>    |
|               | Biological risks                                                   | 5.4.1 <sup>a</sup>   |         | Caution                                             | 5.4.4 <sup>a</sup>   |
|               | Common Units                                                       | Proprietary          |         | Document face up <sup>f</sup>                       | 1952 <sup>e</sup>    |
| YYYY-MM-DD    | Date format (year-month-day)                                       | N/A                  |         | International System of Units                       | Proprietary          |
|               | Target                                                             | Proprietary          | YYYY-MM | Date format (year-month)                            | N/A                  |
|               |                                                                    |                      |         | Interval                                            | Proprietary          |

| Symbol                                                                            | Symbol Title             | Source      | Symbol                                                                            | Symbol Title                                                                                                  | Source      |
|-----------------------------------------------------------------------------------|--------------------------|-------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------|
|  | Handheld barcode scanner | Proprietary |  | Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid. | Proprietary |
|  | Lot details              | Proprietary |  | Master Curve definition                                                                                       | Proprietary |
|  | Calibrator lot value     | Proprietary |  | Quality control lot value                                                                                     | Proprietary |

- <sup>a</sup> International Standard Organization (ISO). ISO 15223-1 Medical Devices- Symbols to be used with medical device labels, labelling and information to be supplied.
- <sup>b</sup> ISO 15223-1:2020-04
- <sup>c</sup> Federal Register. Vol. 81, No 115. Wednesday, June 15, 2016. Rules and Regulations: 38911.
- <sup>d</sup> IVDR REGULATION (EU) 2017/746
- <sup>e</sup> International Standard Organization (ISO). ISO 7000 Graphical symbols for use on equipment.
- <sup>f</sup> Indicates Assay-eNote



Siemens Healthcare Diagnostics Inc.  
511 Benedict Avenue  
Tarrytown, NY 10591 USA

**Siemens Healthineers Headquarters**

Siemens Healthcare GmbH  
Henkestraße 127  
91052 Erlangen  
Germany  
Phone: +49 9131 84-0  
siemens-healthineers.com