Cancer Antigen CA 15-3

ADVIA Centaur XP System

Principle of the Test

The ADVIA Centaur® CA 15-3 assay is a fully automated, two-step sandwich immunoassay using direct, chemiluminescent technology. The Lite Reagent is composed of the monoclonal mouse antibody, DF3, specific for CA 15-3, labeled with acridinium ester. The Conjugate Reagent is composed of the monoclonal mouse antibody 115D8, specific for CA 15-3, labeled with fluorescein. The Solid Phase is composed of purified monoclonal mouse capture antibody, which is covalently coupled to paramagnetic particles. The sample is incubated with both Conjugate Reagent and Solid Phase simultaneously for 20 minutes. After incubation, the immuno-complex is washed and the Lite Reagent is added, incubated for an additional 20 minutes and then washed again. This two-step protocol eliminates high-dose hook effect in this assay.

The system automatically performs the following steps:

- dispenses 20 μL of sample into a cuvette
- dispenses 50 μL of Conjugate Reagent and 250 μL of Solid Phase and incubates for 20 minutes at 37°C
- washes with Wash 1
- resuspends with 250 μL of Wash 1
- dispenses 50 μL of Lite Reagent and incubates for 20 minutes at 37°C
- separates, aspirates and washes the cuvettes with Wash 1
- dispenses 300 μL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

A direct relationship exists between the amount of CA 15-3 present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Clinical Application and Usefulness

The ADVIA Centaur CA 15-3 assay is an in vitro diagnostic test for the quantitative serial determination of cancer antigen CA 15-3 in human serum using the ADVIA Centaur System. When used in conjunction with other clinical and diagnostic procedures, serial testing with the ADVIA Centaur CA 15-3 assay is useful for monitoring the course of disease and therapy in metastatic breast cancer patients, and for detection of recurrence in previously treated Stage II or Stage III breast cancer patients. This assay is not intended for use on any other system.

WARNING: The concentration of CA 15-3 in a given specimen, as determined by assays from different manufacturers, can vary because of differences in assay methods and reagent specificity. The results...
reported by the laboratory to the physician must include the identity of the assay for CA 15-3 used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining serial levels of CA 15-3 is changed, the laboratory must perform additional serial testing to confirm baseline values. The ADVIA Centaur CA 15-3 assay is based on the DF3 and 115D8 antibodies available through agreement with Fujirebio Diagnostics®, Inc.

United States federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

Specimen Collection and Handling

Specimen Collection

⚠️ BIOHAZARD

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum is the recommended sample type for this assay. Gold Top Vacutainer (with SST) is preferred collection tube.
- This assay requires 20 µL of sample for a single determination. Additional volume is required for onboard dilutions. Note: the dead space using the 13x100 mm false bottom tubes is 50 uL.
- Allow samples to clot adequately before centrifugation.
- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

Specimen Storage and Stability

- Keep tubes covered and upright at all times.
- Do not use samples that have been stored at room temperature for longer than 8 hours.
- Tightly cover and refrigerate specimens at 2 to 8°C if the assay is not completed within 8 hours.
- Freeze samples at or below -20°C if the sample is not assayed within 24 hours.
- Freeze samples only once and mix thoroughly after thawing.

Reagents

Storage and Stability

- Store the reagents upright at 2–8 °C.
- Primary reagents stable until the expiration date on the pack label, or for 14 days onboard the system.
• Wash 1 stable until the expiration date on the bottle label, or for 14 days onboard the system.
• Diluent stable until the expiration date on the pack label, or for 28 consecutive days after accessing the ancillary reagent pack.

CAUTION:
• Discard the primary reagent packs at the end of the onboard stability interval.
• Do not use reagents beyond the expiration date.

Ingredients
Reagent ingredients for the ADVIA Centaur CA 15-3 assay are as follows:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lite Reagent</td>
<td>5.0 mL/ReadyPack®</td>
<td>monoclonal mouse anti-DF3 antibody (~ 2.0 µg/mL) labeled with acridinium ester in buffered saline with bovine serum albumin, sodium azide (&lt; 0.1%), and preservatives</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>25.0 mL/ReadyPack</td>
<td>monoclonal mouse capture antibody (~30 µg/mL) covalently coupled to paramagnetic particles in buffer with bovine serum albumin, sodium azide (&lt; 0.1%), preservatives</td>
</tr>
<tr>
<td>Conjugate Reagent</td>
<td>5.0 mL/ReadyPack</td>
<td>monoclonal mouse anti-115D8 antibody (~12.5 µg/mL) labeled with a thiocarbamate of fluorescein in buffered saline with bovine serum albumin and preservatives</td>
</tr>
<tr>
<td>Wash 1</td>
<td>1500 mL/bottle</td>
<td>phosphate buffered saline with sodium azide (&lt; 0.1%) and surfactant</td>
</tr>
<tr>
<td>Multi-Diluent 1</td>
<td>25.0 mL/ReadyPack</td>
<td>equine serum with sodium azide (0.1%) and preservatives</td>
</tr>
</tbody>
</table>

**WARNING:** Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**Reagents Special Preparation**
No special preparation of reagents is required.

Note: All new primary and ancillary reagents must be mixed before placement on the instrument.
See “Set Up and Daily Operations” procedure for mixing instructions.

**Calibration**

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

**Two-point Calibration Interval**

Use CA 15-3 Calibrator to perform two-point calibrations.

Perform a two-point calibration every 14 days. Additionally, calibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

**Defining Calibrator Values for Two-point Calibration**

1. At the workspace, select **Calibration**.
2. Select **Calibrator Definition**.
3. Select **Scan Data**.
4. Scan the barcodes on the Calibrator Assigned Value Card.
5. Define the LIS field.
6. Review the values on the Calibrator Assigned Value card to ensure that they are correct. Scan again, if required.
7. Select **Save**.

**Master Curve**

Use the barcode reader to enter the Master Curve values from the Master Curve card onto the system. Ensure that the lot number on the Master Curve matches the lot number of the ReadyPack.

**Defining the Master Curve Using the Barcode Scanner**

1. At the workspace, select **Calibration**.
2. Select **Master Curve Definition**.
3. At the Calibration-Master Curve Definition window, select **Scan Data**.
4. Scan the barcodes on the CA 15-3 Master Curve Card.
5. Select Save.

**Quality Control (QC)**

BioRad Lyphochek Tumor Marker Control, Levels 1 and 2.

Using a Volumetric pipet, reconstitute each vial of control with 2.0 mL of Reagent water. Allow the controls to stand at room temperature for approximately 15 minutes, swirling occasionally. After reconstitution, the controls are frozen in 0.4 mL aliquots. The frozen aliquots are stable for 30 days at -20°C. See posted QC chart for acceptability limits.

**QC Frequency**

Analyze all levels of quality control material on each day that samples are analyzed.

Analyze all levels of quality control material each time a two-point calibration is performed.

**Troubleshooting Out-of-Range QC Values**

A QC run is acceptable when all values fall within the expected ranges.

If the CA 15-3 QC results do not fall within the defined ranges then the run is rejected, and you must take the following corrective action:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, recalibrate the system and then rerun the quality control samples or contact Bayer Diagnostics for more assistance

**Instrument Operation and System Description**

The ADVIA Centaur system is an automated, random access, direct chemiluminescent immunoassay analyzer that offers no-pause reloading of reagents, samples, and supplies. Comprehensive assay groups are available.

When the sample start button is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Paramagnetic particles are magnetically separated in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Acid and Base. The chemiluminescent reaction occurs in the luminometer. The PMT measures the chemical light reaction that takes place.

Refer to Section 4 in the ADVIA Centaur Reference Manual for detailed procedures that describe how to schedule samples and manage the worklist.

**A. System Start Button**

There is one (1) main system operation button on the ADVIA Centaur, the Start button. Pressing this button performs the following actions:
• Homes the subsystems.
• Starts specimen sampling.
• If the Start button is pressed while the system is processing samples, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

B. Start-up
1. Put the samples racks on the sample entry queue.
2. Press the Start button.

C. Verify Supplies:
While the system is running, you can manage the following supplies without interrupting the run.
• Reagent water supply
• Liquid waste container
• Acid and Base reagents
• Cuvette waste bin
• Sample tip waste bin and tip tray waste area
• Cuvette supply
• Sample tip supply
• Ancillary reagent packs
• Primary reagent packs

D. Scheduling a Run/Entering a Worklist
You can enter a worklist by different methods:

Automated Worklist
You can send the worklist to the ADVIA Centaur from a Lab Information System (LIS). This is done in a unidirectional manner (Dynamic Download) or in a bi-directional manner (Host Query).

Manual (Operator-initiated) Worklist

Scheduling Calibrators
1. At the workspace, select Worklist and then select Schedule.
2. Select the Calibrator box.
3. Select the test you want to schedule for calibration.
4. The reagent lot that is on the system and any defined calibrators are displayed. Select the appropriate reagent and calibrator lot combination.

5. Select Save.

6. Ensure that the lot numbers of reagent and calibrator are available for system use.

Scheduling Controls

1. At the workspace, select Worklist and then select Schedule.
2. Select the Control box.
3. Select each test you would like to schedule.
4. Select each control level and lot number you need to run.
5. Select Save.

Scheduling Patient Samples

1. At the workspace, select Worklist and then select Schedule.
2. Select the Patient box.
3. Enter a patient sample identification number (SID).
4. Press the Enter key.
5. Select each test, profile, dilution, and/or replicates needed.
6. Select Save.

For detailed operating procedural information, refer to the ADVIA Centaur Reference Manual or to the online help system.

E. Loading Sample Racks

BIOHAZARD

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Gently mix the calibrators and quality controls before dispensing into the sample cups.
2. Load the sample cups containing the calibrators, controls, or patient specimen into any Centaur rack.
3. Load racks onto the sample entry queue.
4. Press the Start button.

F. Loading Ancillary Reagents

Place the ancillary reagent pack on the ancillary entry queue until the green light comes on. The ADVIA Centaur moves the pack into the ancillary queue.

G. Loading Primary Reagents

**CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing reagents for use, refer to Appendix C, *Handling Reagents*, in your ADVIA Centaur Assay Manual.

1. Gently mix the CA 15-3 ReadyPack before placing it on the reagent shelf for the first time.
2. Load the CA 15-3 ReadyPack by matching the color on the pack to the color-coded reagent shelf.
3. Close the reagent compartment door.

H. Loading STAT Samples in the STAT Entry Queue

1. Press the Start button, if the green Start light is not lit.
2. Place the STAT sample tube or sample cup in the rack.
3. Load the rack in the STAT entry queue until the green STAT light comes on, indicating the system has accepted the rack.

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**Performance Characteristics**

**Precision**

Eight samples were assayed 3 times, in 10 runs, on 1 system (n = 30 for each sample), over a period of 5 days. The total % CV had a range of 3.0 to 4.3%. The within-run % CV was under 3.7% for all samples. The following table is a summary of the results.

<table>
<thead>
<tr>
<th>Mean</th>
<th>Within-run %CV</th>
<th>Within-day %CV</th>
<th>Total %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0</td>
<td>3.7</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>18.4</td>
<td>3.6</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td>21.9</td>
<td>2.4</td>
<td>2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>34.5</td>
<td>2.8</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>35.9</td>
<td>3.2</td>
<td>2.2</td>
<td>4.0</td>
</tr>
<tr>
<td>45.5</td>
<td>3.1</td>
<td>0.0</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Specificity

There are no known cross-reactants for CA 15-3. The potential interference of chemotherapeutic agents was tested by adding these substances to a serum pool containing 37 U/mL of CA 15-3. The chemotherapeutic agents were spiked into this serum pool to a final concentration listed in parenthesis. These spiked pools were assayed for CA 15-3 and percent recovery to the unspiked pool was calculated.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mean % Recovery</th>
<th>Substance</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatinum (175 μg/mL)</td>
<td>97.5</td>
<td>Herceptin (400 μg/mL)</td>
<td>92.6</td>
</tr>
<tr>
<td>Cyclophosphamide (800 μg/mL)</td>
<td>96.0</td>
<td>Mitomycin C (75 μg/mL)</td>
<td>98.1</td>
</tr>
<tr>
<td>Diethylstilbestrol (25 μg/mL)</td>
<td>100.7</td>
<td>Megestrol acetate (10 μg/mL)</td>
<td>99.8</td>
</tr>
<tr>
<td>Doxorubicin HCl (50 μg/mL)</td>
<td>97.5</td>
<td>Methotrexate (450 μg/mL)</td>
<td>104.1</td>
</tr>
<tr>
<td>Etoposide (10 μg/mL)</td>
<td>96.0</td>
<td>Tamoxifen (80 μg/mL)</td>
<td>99.7</td>
</tr>
<tr>
<td>S-Phenylurea (1 μg/mL)</td>
<td>99.8</td>
<td>Vincristine (1.5 μg/mL)</td>
<td>97.0</td>
</tr>
<tr>
<td>Flumethadine (10 μg/mL)</td>
<td>100.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interference testing was determined according to NCCLS Document EP7-P.15

Analytical Sensitivity

The ADVIA Centaur CA 15-3 assay has an analytical sensitivity of 0.50 U/mL.

Analytical sensitivity is defined as the concentration of CA 15-3 that corresponds to the RLUs that are two standard deviations more than the mean RLUs of replicate determinations of the CA 15-3 zero standard. This response is an estimate of the minimum detectable concentration with 95% confidence.

Analytical Measuring Range (AMR)

The analytical measuring range of the ADVIA Centaur CA 15-3 assay is 0.50 to 200 U/mL.

Reportable Range

Values below 1 U/mL are reported as <1 U/mL. Values above 200 U/mL are run on dilution until an answer is achieved.

Dilutions

- Serum samples with levels of CA 15-3 greater than 200 U/mL must be diluted and retested to obtain accurate results.
• Patient samples can be automatically diluted by the system or prepared manually.

• The Centaur is programmed to rerun samples that exceed 200 U/mL on a 1:20 instrument performed dilution. You will need to place the sample rack back onto the loading queue and press start (unless green light is on). Note: the Centaur will report a value that has already been corrected for the dilution.

• If the 1:20 dilution is not high enough, then manually perform a dilution using Multi-Diluent 1 until an answer is achieved. **Important:** you will need to mathematically correct for the manual dilution you performed to report out the diluted result.

• For automatic dilutions, ensure that ADVIA Centaur Multi-Diluent 1 is loaded and set the system parameters as follows
  
  - Dilution point: \( \leq 200 \text{ U/mL} \)
  - Dilution factor: 5, 10, 20

  For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.

**Reference Interval**

Less than 33 U/mL for females. Male reference ranges are not available.

**Units for Reporting Results**

The system reports serum CA 15-3 results in U/mL. Units are user-defined in the system software. CA15-3 values are ported in whole numbers.

**Procedure Notes**

**Interchangeability of Assay Values**

The concentration of CA 15-3 in a given specimen can vary due to differences in assay methods and reagent specificity. Do not use values obtained with different CA 15-3 assay methods interchangeably.

**Disposal**

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 all federal, state, and local requirements.
Method Limitations

Do not interpret levels of CA 15-3 as absolute evidence of the presence or the absence of malignant disease. Measurements of CA 15-3 should always be used in conjunction with other diagnostic procedures.

Do not use the ADVIA Centaur CA 15-3 assay as a screening test or for diagnosis. Normal levels of CA 15-3 do not always preclude the presence of disease.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. 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.

Evidence suggests that patients undergoing retinal fluorescein angiography can retain amounts of fluorescein in the body for up to 36 to 48 hours post-treatment. In the cases of patients with renal insufficiency, including many diabetics, retention could be much longer. Such samples can produce either falsely elevated or falsely depressed values when tested with this assay, and should not be tested.

Exercise caution when interpreting ADVIA Centaur CA 15-3 levels during pregnancy.

The concentration of CA 15-3 in a given specimen can vary because of differences in assay methods, calibration, and reagent specificity. CA 15-3 determined with different manufacturers’ assays will vary depending on the method of standardization and antibody specificity. Use assay-specific values to evaluate quality control results.

Endogenous interferents, such as high concentrations of hemoglobin, lipids, bilirubin and total protein, had no effect beyond the limits of random within-run variations. Mean recoveries were within 5% of the control level for all interferents tested.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount Added</th>
<th>LX20 Index</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugated Bilirubin</td>
<td>25 mg/dL</td>
<td>&gt;20</td>
<td>95.3</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>900 mg/dL</td>
<td></td>
<td>106.2</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1000 mg/dL</td>
<td>&gt;10</td>
<td>96.9</td>
</tr>
<tr>
<td>Total Protein</td>
<td>6.5 g/dL</td>
<td></td>
<td>101.0</td>
</tr>
</tbody>
</table>

If there is an interferant (hemolysis, lipemia and icterus), that exceeds the values in the above table, run the sample undiluted and append the corresponding ETC Code:

- **TUR** “Specimen turbid, result may be invalid”
- **ICTRQ** “Specimen icteric, result may be invalid”
- **HEMRQ** “Specimen hemolyzed, result may be invalid”
For additional information on performance characteristics including cross reactivity and dilution recovery, see the product information in the ADVIA Centaur Assay Manual.

**Equipment and Supplies**

- ADVIA Centaur CA 15-3 ReadyPack
- ADVIA Centaur CA15-3 Calibrator
- ADVIA Centaur Wash 1
- ADVIA Centaur Multi-Diluent 1
- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- ADVIA Centaur Cleaning Solution Concentrate
- ADVIA Centaur Acid Reagent (0.5% H$_2$O$_2$, 0.1N HNO$_3$)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

**References**

1. Bayer Diagnostics ADVIA Centaur CA 15-3 product insert, Revision A.
2. Bayer Diagnostics ADVIA Centaur Reference Manual, Revision D.

**Technical Assistance**

Bayer Diagnostics Technical Care Center: 1-877-229-3711  
Customer Service: 1-800-255-3232  
Serial Number: IRL80600727  
Customer Account Number: 139214