Carcinoembryonic Antigen (CEA)

*ADVIA Centaur System*

**Principle of the Test**

The ADVIA Centaur® CEA assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a purified polyclonal rabbit anti-CEA antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-CEA antibody covalently coupled to paramagnetic particles.

The system automatically performs the following steps:

- dispenses 50 µL of sample into a cuvette
- dispenses 50 µL of Lite Reagent and 250 µL of Solid Phase and incubates for 7.5 minutes at 37°C
- separates, aspirates, and washes the cuvettes with reagent water
- 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

**Clinical Application and Usefulness**

For *in vitro* diagnostic use in the quantitative measurement of carcinoembryonic antigen (CEA) in serum to aid in the management of cancer patients in whom changing concentrations of CEA are observed using the ADVIA Centaur System.

**WARNING:** The concentration of CEA in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CEA assay 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 CEA levels is changed, the laboratory must perform additional serial testing to confirm baseline values.

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 50 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 48 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.

• 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 CEA 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>polyclonal rabbit anti-CEA antibody (~400 ng/mL) labeled with acridinium ester in phosphate buffered saline with protein stabilizers, sodium azide (0.12%), and preservatives</td>
</tr>
</tbody>
</table>
Solid Phase  25.0 mL/ReadyPack  monoclonal mouse anti-CEA antibody (~120 µg/mL) covalently coupled to paramagnetic particles in phosphate buffered saline with protein stabilizers, sodium azide (0.11%), and preservatives

CEA Diluent  5.0 mL/ReadyPack  bicine buffer, gelatin, and BSA with preservatives and sodium azide (0.1%)

**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](image)

**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**

Use this procedure to mix ADVIA Centaur CEA primary reagent packs that are unpierced.

**CAUTION:** Do not use this procedure for pierced ADVIA Centaur CEA reagent packs. Discard pierced ADVIA Centaur CEA reagent packs that have been removed from the system.

1. Hold the reagent pack firmly with thumb on one side and fingers on the other side. Shake vigorously for 15 seconds, using a back and forth motion.
2. Hold the reagent pack firmly at one end, with film side up, and tap sharply on a bench top five times to reduce foaming caused by shaking.

**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 Calibrator D to perform two-point calibrations.

Perform a two-point calibration every 21 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 CEA Master Curve Card.

5. Select Save.

**Quality Control (QC)**

BioRad Immunoassay Plus Control 1, 2 & 3

Each control is reconstituted with 5.0 mL of CLRW (Clinical Laboratory Reagent Water). The reconstituted controls are stable for 7 days when stored at 2-8 °C.

See posted QC chart for acceptability limits.

**QC Frequency**

Analyze all 3 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 CEA 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 CEA ReadyPack before placing it on the reagent shelf for the first time.
2. Load the CEA 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.

**Performance Characteristics**

**Precision**

Four samples were assayed 6 times, in each of 12 runs, on 5 systems, (n = 72 for each sample), over a period of 4 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (ng/mL) (μg/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>3.6</td>
<td>4.1</td>
<td>5.5</td>
</tr>
<tr>
<td>14.2</td>
<td>3.3</td>
<td>2.9</td>
<td>4.4</td>
</tr>
<tr>
<td>31.5</td>
<td>2.8</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td>51.5</td>
<td>2.1</td>
<td>2.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Traceability of Standardization**

The ADVIA Centaur CEA assay is traceable to an internal standard manufactured using highly purified material. Assigned values of calibrators and ranges of Tumor Marker Plus and Ligand Plus controls are traceable to this standardization.
**Specificity**

The potential interference of NCA (normal cross-reacting antigen) and NCA2 was tested by adding these antigens to serum pools containing CEA. The level of CEA was then determined.

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>CEA value without cross-reactant (ng/mL) (µg/L)</th>
<th>CEA value with cross-reactant (ng/mL) (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCA (500 ng/mL)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>23.3</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>71.2</td>
<td>66.1</td>
</tr>
<tr>
<td>NCA2 (100 ng/mL)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>23.3</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>71.2</td>
<td>62.0</td>
</tr>
</tbody>
</table>

NCA and NCA2 showed minimal interference with the recovery of CEA from the serum samples. Average recovery is greater than 95%.

**Interference by Chemotherapeutic Agents**

The potential interference of chemotherapeutic agents was tested by adding these agents to serum pools containing CEA. The level of CEA in each of these pools was then determined and normalized to the level without the respective drugs.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount Added (µg/mL)</th>
<th>Mean % Recovery (Spike/control x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincaistine</td>
<td>0.70</td>
<td>100.4</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>1.20</td>
<td>99.2</td>
</tr>
<tr>
<td>Cisplatinum</td>
<td>1.50</td>
<td>97.9</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>133</td>
<td>98.9</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>3300</td>
<td>96.9</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>360</td>
<td>96.9</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>100</td>
<td>96.9</td>
</tr>
<tr>
<td>Folinic Acid</td>
<td>60</td>
<td>98.4</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>60</td>
<td>96.6</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>4500</td>
<td>98.7</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>1300</td>
<td>100.9</td>
</tr>
</tbody>
</table>

Interference testing was determined according to NCCLS Document EP7-P.12

**Analytical Sensitivity**

The ADVIA Centaur CEA assay has an analytical sensitivity of 0.5 µg/L (1.08 IU/mL).
Sensitivity was determined by diluting low patient specimens with the zero standard. The sensitivity of the assay with a particular patient specimen was taken as that concentration which was statistically different from both the zero standard and the next lowest dilution of patient sample.

*Analytical Measuring Range (AMR)*

The analytical measuring range of the ADVIA Centaur is 0.5 µg/L to 100 µg/L.

*Reporting Results*

*Reportable Range:*

Results below 0.5 µg/L are reported as <0.5 µg/L. Results above 100 µg/L are run on dilution until an answer is achieved.

*Dilutions*

- Serum samples with CEA levels greater than 100 µg/L (ng/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 100 µg/L on a 1:50 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 1:50 dilution is not high enough, order a 1:100 onboard dilution. Note: the Centaur will report a value that has already been corrected for the dilution.
- If the 1:100 value is not high enough, then manually perform a dilution using CEA diluent until an answer is achieved. **Important:** you will need to correct for the manual dilution you performed to report out the diluted result.
- For automatic dilutions, ensure that ADVIA Centaur CEA Diluent is loaded and set the system parameters as follows:
  - Dilution point: ≤ 100 µg/L (ng/mL)
  - Dilution factor: 5, 10, 50, 100
- For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.

*Reference Interval*

- Less than 3.8 µg/L (ng/mL)
**Expected Results**

The expected results for the ACS:180® CEA assay were previously established. Data was obtained as shown in the following table. Serum samples from healthy subjects and patients with various malignant diseases were analyzed. The cancer patients included in this study represented a variety of disease states from active, progressive malignancy to no clinical evidence of disease. The frequency of positive CEA results was significantly lower in patients with no evidence of active disease compared to those with active disease.

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>N</th>
<th>0-2.5</th>
<th>2.6-5.0</th>
<th>5.1-10.0</th>
<th>10.1-20</th>
<th>&gt; 20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>225</td>
<td>98.2</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smokers</td>
<td>150</td>
<td>87.3</td>
<td>8.0</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Units for Reporting Results**

The system reports serum CEA results in µg/L (SI units) or ng/mL (mass units). The conversion formula is 1 µg/L = 1 ng/mL. Units are user-defined in the system software.

**Procedure Notes**

**Calculations**

For detailed information about how the system calculates results, refer to the ADVIA Centaur Reference Manual or to the online help system.

**High Dose Hook Effect**

Patient samples with high CEA levels can cause a paradoxical decrease in the RLUs (high dose hook effect). In this assay, patient samples with CEA levels as high as 100,000 µg/L (ng/mL) will assay greater than 100 µg/L (ng/mL).

**Interchangeability of Assay Values**

The concentration of CEA in a given specimen can vary due to differences in assay methods and reagent specificity. Do not use values obtained with different 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 CEA as absolute evidence of the presence or the absence of malignant disease. Measurements of CEA should always be used in conjunction with other diagnostic procedures.

The concentration of CEA in a given specimen can vary due to differences in assay methods, calibration, and reagent specificity. CEA determined with different manufacturers’ assays will vary depending on the method of standardization and antibody specificity.

Do not use the ADVIA Centaur CEA immunoassay as a screening test for diagnosis.

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.

**Serum specimens that are . . .**

<table>
<thead>
<tr>
<th>Demonstrate ≤ 5% change in results up to . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolyzed</td>
</tr>
<tr>
<td>lipemic</td>
</tr>
<tr>
<td>icteric</td>
</tr>
</tbody>
</table>

If there is an interferant (hemolysis, lipemia and icterus), that exceeds the values above, then 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 CEA ReadyPack
• ADVIA Centaur Calibrator D
• ADVIA Centaur CEA Diluent
• 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 CEA product insert, Revision C.
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: IRL 80600727
Customer Account Number: 139214