**Vitamin B<sub>12</sub>**

**ADVIA Centaur System**

**Principle of the Test**

The ADVIA Centaur<sup>®</sup> VB12 assay is a competitive immunoassay using direct chemiluminescent technology in which vitamin B<sub>12</sub> from the patient sample competes with vitamin B<sub>12</sub> labeled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase. The assay uses Releasing Agent (sodium hydroxide) and DTT to release the vitamin B<sub>12</sub> from the endogenous binding proteins in the sample and cobinamide to prevent rebinding after the Solid Phase is added to the sample.

The system automatically performs the following steps:

- washes the ancillary reagent probe with 100 µL of T3/T4/VB12 Ancillary Reagent
- dispenses 100 µL of sample into a cuvette
- dispenses 115 µL of DTT/Releasing Agent
- dispenses 200 µL of Solid Phase and incubates for 5.0 minutes at 37°C
- dispenses 200 µL of Lite Reagent and incubates for 2.5 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

An inverse relationship exists between the vitamin B<sub>12</sub> in the sample and the relative light units (RLUs) detected by the system.

**Clinical Application and Usefulness**

For *in vitro* diagnostic use in the quantitative determination of vitamin B<sub>12</sub> in serum or plasma using the ADVIA Centaur System.

**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.
• This assay requires 100 µL of sample for a single determination. Additional volume is required for onboard dilutions.
• Allow serum samples to clot adequately before centrifugation.
• Samples are free of fibrin or other particulate matter.
• Samples are free of bubbles.

Specimen Storage and Stability
• Do not use samples that have been stored at room temperature for longer than 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.

Specimen Rejection Criteria
Unlabelled specimens are rejected
Grossly hemolyzed samples are rejected

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 28 days onboard the system.
• DTT/Releasing Agent stable for 108 hours (4.5 days) after preparation; do not use beyond this date.
• Ancillary reagents stable until the expiration date on the pack label, or for 14 consecutive days after accessing the ancillary reagent pack.
• 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. Discard the DTT/Releasing Agent ancillary reagent pack after 108 hours. Discard the T3/T4/VB12 Ancillary Reagent ancillary reagent pack after 14 days.
• Do not use reagents beyond the expiration date.

Ingredients
Reagent ingredients for the ADVIA Centaur VB12 assay are as follows:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Ingredients</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lite Reagent</td>
<td>20.0 mL/ReadyPack®</td>
<td>acridinium ester-labeled vitamin B₁₂ (~3 ng/mL) in buffer with sodium azide (0.1%), protein stabilizers, and preservatives</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>20.0 mL/ReadyPack</td>
<td>purified hog intrinsic factor (~0.025 µg/mL) covalently coupled to paramagnetic particles in buffer with sodium azide (0.1%), protein stabilizers, cobinamide, and preservatives</td>
</tr>
<tr>
<td>Releasing Agent</td>
<td>25.0 mL/vial</td>
<td>sodium hydroxide (~0.30N) with potassium cyanide (~1.25 mg/vial)</td>
</tr>
<tr>
<td>DTT</td>
<td>2.0 mL/vial</td>
<td>dithiothreitol (~210 mg/vial)</td>
</tr>
<tr>
<td>T3/T4/VB12 Ancillary Reagent</td>
<td>25.0 mL/ReadyPack</td>
<td>0.4N sodium hydroxide</td>
</tr>
<tr>
<td>VB12 Diluent</td>
<td>5.0 mL/ReadyPack</td>
<td>buffered HSA with sodium azide (0.2%) 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**

**Prepare DTT/Releasing Agent, as follows:**

**CAUTION:** Irritant of the eyes and skin. In case of contact with the eyes or skin, wash immediately and copiously with water and then contact a physician.

1. Add 300 µL DTT to 12.0 mL Releasing Agent in a test tube using a volumetric pipet.
2. Mix the DTT and Releasing Agent in the test tube. Cover the test tube with self-sealing laboratory film and invert the test tube several times to mix.
3. Remove the self-sealing laboratory film and pour the entire contents into the disposable ancillary reagent pack provided.
4. Place a pack seal on the disposable ancillary reagent pack. Ensure that the seal is centered over the opening of the pack, and press firmly on the adhesive portion of the seal.

**NOTE:** Careful preparation of DTT/Releasing Agent is required to obtain accurate and consistent results since the absolute amount of DTT delivered for each test can affect results. Prepare the DTT/Releasing Agent immediately before using. Use the prepared DTT/Releasing Agent within 108 hours (4.5 days).
**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 C to perform two-point calibrations.

Perform a two-point calibration every 28 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 VB12 Master Curve Card.
5. Select **Save**.
**Quality Control (QC)**

**BioRad Lyphochek Anemia control.**

Reconstitute with 3.0 mL CLRW (Clinical Laboratory Reagent Water). The reconstituted control is stable for 14 days when stored at 2-8 °C.

**BioRad Immunoassay Plus Control** levels 1 & 3

Each control is reconstituted with 5.0 mL of CLRW. 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 three levels of quality control material on each day that samples are analyzed.

Analyze all three 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 VB12 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 VB12 ReadyPack before placing it on the reagent shelf for the first time.
2. Load the VB12 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 6 systems, (n = 72 for each sample), over a period of 3 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (pg/mL)</th>
<th>Mean (pmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>178.76</td>
<td>131.89</td>
<td>5.0</td>
<td>9.2</td>
<td>10.4</td>
</tr>
<tr>
<td>207.22</td>
<td>152.89</td>
<td>4.0</td>
<td>4.4</td>
<td>5.9</td>
</tr>
<tr>
<td>608.83</td>
<td>449.19</td>
<td>2.7</td>
<td>2.7</td>
<td>3.8</td>
</tr>
<tr>
<td>1343.87</td>
<td>991.51</td>
<td>2.4</td>
<td>3.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Standardization

The ADVIA Centaur VB12 assay is traceable to an internal standard manufactured using U.S.P. (United States Pharmacopeia) material. Assigned values for calibrators and ranges of Ligand Plus controls are traceable to this standardization.

Specificity

The cross-reactivity of the ADVIA Centaur VB12 assay with cobinamide was determined by adding 20 ng/mL of cobinamide to samples containing 0, 250, and 1000 pg/mL (0, 184, and 738 pmol/L) of vitamin B12. No interference was found at these levels.

Interference testing was determined according to NCCLS Document EP7-P.10

Analytical Sensitivity

The ADVIA Centaur Vitamin B12 assay has an analytical sensitivity of 45 pg/mL. Analytical sensitivity is defined as the concentration of vitamin B12 that corresponds to the RLUs that are two standard deviations less than the mean RLUs of 20 replicate determinations of the Vitamin B12 zero standard.

Analytical Measuring Range

The analytical measuring range of the Centaur Vitamin B12 assay is 45 to 2000 pg/mL.
Reportable Range

The reportable range of the ADVIA Centaur VB12 assay is 45 pg/mL (33 pmol/L) to 2000 pg/mL (1476 pmol/L). Dilutions are not performed. Results below 45 pg/mL are reported as <45 pg/mL. Results above 2,000 are reported as >2000 pg/mL.

**All patient samples with a Vitamin B12 result less than 100 pg/mL should be repeated before reporting the result.**

Dilutions

Dilutions are not performed

Reference Interval

The expected results for the ACS:180® VB12 assay were previously established. Data was obtained on 298 serum samples, including 253 samples from apparently healthy individuals and 45 samples from physician-diagnosed vitamin B12 deficient patients. Ninety-five percent of the values for the apparently healthy individuals fell in the range of 211 to 911 pg/mL (156 to 672 pmol/L).

<table>
<thead>
<tr>
<th>Category</th>
<th>Median (pg/mL)</th>
<th>Range (pg/mL)</th>
<th>Median (pmol/L)</th>
<th>Range (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>382</td>
<td>211-911</td>
<td>282</td>
<td>156-672</td>
</tr>
<tr>
<td>Deficient</td>
<td>160</td>
<td>32-246</td>
<td>118</td>
<td>24-181</td>
</tr>
</tbody>
</table>

**Units for Reporting Results**

The system reports vitamin B12 results in pg/mL (mass units) or pmol/L (SI units). The conversion formula is 1 pg/mL = 0.7378 pmol/L. 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.

**DTT/Releasing Agent Barcode Labels**

DTT/Releasing Agent barcode labels are lot number specific. Do not use barcode labels from one lot of DTT/Releasing Agent with any other lot of DTT/Releasing Agent.
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
Preservatives, such as fluoride and ascorbic acid interfere with the ADVIA Centaur VB12 assay.
Excessive exposure to light may alter vitamin B₁₂ values.
Dispose of the DTT/Releasing Agent ancillary reagent pack after 108 hours.
A comparison of values for 207 pairs of serum and plasma specimens in the range of 147 to 1033 pg/mL (108 to 762 pmol/L) yielded the following regression equation:

\[ \text{plasma VB12} = 1.03 \times (\text{serum VB12}) + 12 \text{ pg/mL}, \ r = 0.96 \]

<table>
<thead>
<tr>
<th>Specimens that are . . .</th>
<th>Have an insignificant effect on the assay up to . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolyzed</td>
<td>150 mg/dL of hemoglobin</td>
</tr>
<tr>
<td>lipemic</td>
<td>3000 mg/dL of triglycerides</td>
</tr>
<tr>
<td>icteric</td>
<td>20 mg/dL of bilirubin</td>
</tr>
</tbody>
</table>

If there is an interferant (hemolysis, lipemia and icterus) higher than the values listed in the above table, run the sample undiluted and send 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 VB12 ReadyPack
- ADVIA Centaur VB12 DTT/Releasing Agent ReadyPack
- ADVIA Centaur T3/T4/VB12 Ancillary Reagent ReadyPack
- ADVIA Centaur Calibrator C
- ADVIA Centaur VB12 Diluent
- ADVIA Centaur Sample Cups and Caps
• ADVIA Centaur Cuvettes
• ADVIA Centaur Tips
• ADVIA Centaur Cleaning Solution Concentrate
• ADVIA Centaur Acid Reagent (0.5% H₂O₂, 0.1N HNO₃)
• ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
• Reagent Water

References

1. Bayer Diagnostics ADVIA Centaur VB12 product insert, Revision J.
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: 619504