

Technical SOP

<b>Title</b>	<b>B-type Natriuretic Peptide (BNP) by ADVIA Centaur CP</b>	
<b>Prepared by</b>	Ashkan Chini	Date: 5/1/2012
<b>Owner</b>	Jean Buss, Robert SanLuis	Date: 5/1/2012

<b>Laboratory Approval</b>		<b>Local Effective Date:</b>
Print Name and Title	Signature	Date
<i>Refer to the electronic signature page for approval and approval dates.</i>		

<b>Annual Review</b>		
Print Name	Signature	Date

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**1. TEST INFORMATION**

<b>Assay</b>	<b>Method/Instrument</b>	<b>Local Code</b>
B-type Natriuretic Peptide	ADVIA Centaur CP	BNP

<b>Synonyms/Abbreviations</b>
BNP

<b>Department</b>
Chemistry

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**2. ANALYTICAL PRINCIPLE**

The ADVIA Centaur BNP assay is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of two monoclonal antibodies. The first antibody, in the Lite Reagent, is an acridinium ester labeled monoclonal mouse anti-human BNP F(ab') fragment specific to the ring structure of BNP. The second antibody, in the Solid Phase, is a biotinylated monoclonal mouse anti-human antibody specific to the C-terminal portion of BNP, which is coupled to streptavidin magnetic particles.

**3. SPECIMEN REQUIREMENTS**

**3.1 Patient Preparation**

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

**3.2 Specimen Type & Handling**

Criteria	
Type -Preferred -Other Acceptable	Plasma (K2 EDTA) None
Collection Container	Lavender Top Tube
Volume - Optimum - Minimum	Full Tube 1 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature, <b>as BNP is unstable in glass containers.</b>
Stability & Storage Requirements	Room Temperature: Not recommended
	Refrigerated: 2-8° C 24 hours
	Frozen: -70° C N/A
Timing Considerations	After centrifugation, store separated plasma samples at 2 - 8° C until testing.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for “test not performed” message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.

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Criteria	
<b>Compromising Physical Characteristics</b>	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
<b>Other Considerations</b>	Centrifuge samples at 1000 g for at least 15 minutes

#### 4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering “SAFETY” for additional information.

##### 4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
ADVIA Centaur BNP	Siemens reagent cartridge Cat. No. 02816634
ADVIA Centaur Multi-Diluent 1	Siemens reagent cartridge Cat. No. 110312
ADVIA Centaur R1 Acid Reagent	Siemens reagent Cat. No. 00497043
ADVIA Centaur R2 Base Reagent	Siemens reagent Cat. No. 00497043
ADVIA Centaur Wash 1 Solution	Siemens reagent Cat. No. 112351
ADVIA Centaur CSC	Siemens reagent Cat. No. 112748

##### 4.2 Reagent Preparation and Storage

**NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.**

**Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.**

Assay Kit: ADVIA Centaur BNP Ready Pack (primary reagent pack)	
<b>Reagent a</b>	Lite Reagent (Volume 10.5 mL)
<b>Reagent b</b>	Solid Phase (Volume 21 mL)
<b>Manufacturer’s Information</b>	Centaur CP BNP Master Curve Cards
<b>Storage</b>	Store the reagent up right at 2 - 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 41.6 days.
<b>Preparation</b>	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.

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<b>Reagent</b>	<b>ADVIA Centaur Multi-Diluent 1</b> (ancillary reagent pack)
<b>Container</b>	Reagent Cartridge
<b>Storage</b>	Store the reagent up right at 2 - 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 28 days.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur R1 Acid Reagent &amp; R2 Base Reagent</b>
<b>Container</b>	Reagent bottle (300 mL)
<b>Storage</b>	Store at 2 - 25° C. Use at 18 - 30° C.
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 30 days. Avoid exposure to light.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur Wash 1 Solution</b>
<b>Container</b>	Reagent bottle (1500 mL)
<b>Storage</b>	Store at 2 - 25° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 30 days.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur Cleaning Solution Concentrate</b>
<b>Container</b>	Reagent bottle (70 mL)
<b>Storage</b>	Store at 2 - 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Prepared reagent is stable for 7 days.
<b>Preparation</b>	<ol style="list-style-type: none"> <li>Carefully pour a container of ADVIA Centaur CP Cleaning Solution Concentrate into the cleaning bottle.</li> <li>Add enough reagent grade water to the bottle or container to bring the total volume of cleaning solution to 2 liters.</li> </ol>

## 5. CALIBRATORS/STANDARDS

### 5.1 Calibrators/Standards Used

<b>Calibrator</b>	<b>Supplier and Catalog Number</b>
Low and High Calibrator	Siemens BNP calibrator, Cat. No. 02817266

## 5.2 Calibrator Preparation and Storage

**NOTE:** Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	BNP Calibrator (Low & High)
Preparation	<ul style="list-style-type: none"> <li>• Add 2.0 mL of reagent grade water into each calibrator vial.</li> <li>• Let the calibrators stand for 15 to 20 minutes at room temperature (20 - 30° C) to allow the lyophilized material to dissolve.</li> <li>• Gently swirl and invert the vials until homogeneous.</li> </ul>
Storage/Stability	<ul style="list-style-type: none"> <li>• Store at 2 - 8° C</li> <li>• <b>Unopened Calibrator</b> is stable until the expiration date on the vial.</li> <li>• <b>Opened Calibrator:</b> once reconstituted the calibrator is stable for 60 days when stored at ≤ -20° C.</li> <li>• <b>Opened Calibrator:</b> once reconstituted the calibrator is stable for 5 days at 2 – 8° C.</li> <li>• <b>Opened Calibrator:</b> once reconstituted the calibrator is stable for 8 hours onboard.</li> </ul>

## 5.3 Calibration Procedure

Criteria	Special Notations
Frequency	<ul style="list-style-type: none"> <li>• When the calibration interval expires</li> <li>• Calibration Interval is every 42 days</li> <li>• A calibration is invalid</li> <li>• New lot or shipment of assay reagents</li> <li>• Controls are repeatedly out of range.</li> </ul>
Procedure	<ol style="list-style-type: none"> <li>1. Enter the calibrator information provided on the ADVIA Centaur CP side of the calibrator assignment value card. Ensure you use the ADVIA Centaur CP side of the card.</li> <li>2. Enter the Master Curve information from the cards provided with the primary reagent packs. Ensure you use the ADVIA Centaur CP side of the card.</li> <li>3. Load the low and high calibrators into appropriate sample pour-off tubes that accommodate the Siemens-supplied barcode label.</li> <li>4. Attach the Siemens-supplied barcode labels to the pour off tubes.</li> </ol>

	<ol style="list-style-type: none"> <li>5. Load the sample tubes into a sample rack. Move the tube-type selector on the rack to position A.</li> <li>6. Load the rack in the sample compartment.</li> <li>7. On the Sample Compartment screen, select the lane containing the sample rack with the calibration material.</li> <li>8. Confirm or enter the calibrator information.</li> <li>9. Select <b>Close</b> to return to the workspace.</li> <li>10. At the workspace, select the primary reagent area.</li> <li>11. Select the assay to calibrate.</li> <li>12. Select <b>Calibrate</b>. The system automatically begins sampling the calibrator material.</li> </ol> <p><b>NOTE:</b> The Calibrate button is not active on the Primary Reagent Screen in the following situations:</p> <ul style="list-style-type: none"> <li>• there are no calibrators defined</li> <li>• there are no Master Curves defined</li> <li>• there is no reagent onboard</li> <li>• the calibration material for defined lots is expired</li> <li>• there is no calibrator material in the sample compartment</li> </ul> <ol style="list-style-type: none"> <li>13. Select <b>Close</b> to return to the workspace.</li> <li>14. To check the time due for the calibration:                     <ol style="list-style-type: none"> <li>a. At the workspace, select <b>Results</b>.</li> <li>b. Select the Calibrations tab to view the Time Due stamp for the assay you are calibrating.</li> <li>c. Select <b>Close</b>.</li> </ol> </li> </ol>
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### 5.5 Tolerance Limits

IF.....	THEN.....
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

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## 6. QUALITY CONTROL

### 6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Cardiac Markers Plus Control levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 181, 182 and 183

### 6.2 Control Preparation and Storage

**NOTE:** Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Liquichek™ Cardiac Markers Plus Control levels 1, 2 and 3
<b>Preparation</b>	<ul style="list-style-type: none"> <li>Allow the frozen control to thaw at room temperature (18-25°C) for 30 minutes or until completely thawed prior to use.</li> <li>Before sampling, gently swirl the vials several times to ensure homogeneity.</li> <li>After each use, promptly replace the stopper and return to 2 - 8°C storage.</li> </ul>
<b>Storage/Stability</b>	<ul style="list-style-type: none"> <li>Store at -20 to -70° C</li> <li><b>Unopened Quality Control</b> is stable until the expiration date on the vial.</li> <li><b>Opened Quality Control:</b> is stable for 15 days when stored tightly capped at 2 - 8° C.</li> </ul>

### 6.3 Frequency

Analyze all 3 levels of QC material after each calibration. Each day of patient testing QC Levels 1 and 3 are run on day shift, Level 2 on evening shift, and either Level 1 or 3 on night shift.

### 6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the Laboratory Information System (LIS), and may be posted near the instrument for use during computer downtime.
2	<b>Run Rejection Criteria</b> <ul style="list-style-type: none"> <li>Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported.</li> </ul>



Step	Action
	<ul style="list-style-type: none"> <li>The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.</li> </ul>
3	<p><b>Corrective Action:</b></p> <ul style="list-style-type: none"> <li>All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be <u>reanalyzed according to the Laboratory QC Program</u>. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program.</li> <li>Corrective action documentation must follow the Laboratory Quality Control Program.</li> </ul>
4	<p><b>Review of QC</b></p> <ul style="list-style-type: none"> <li>QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee.</li> <li>If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.</li> </ul>

IF the Quality Control ...	THEN...
does not fall within the Expected Values	<ul style="list-style-type: none"> <li>Verify that the materials are not expired.</li> <li>Verify that required maintenance was performed.</li> <li>Verify that the assay was performed according to the instructions for use.</li> <li>Rerun the assay with fresh quality control samples.</li> <li>If necessary recalibrate the assay and repeat quality control</li> <li>If unable to resolve issue and/or instrument is inoperable, testing may be performed on alternative method B-type Natriuretic Peptide (BNP) by Triage Meter. Refer to one of the appropriate SOPs: WAH.H11, SGAH.H11, or GEC.H08 for instructions.</li> </ul>

### 6.5 Review Patient Data

Technologist must review each result print-out for error messages. Refer to the Centaur Operator's Guide for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

## 6.6 Documentation

- QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

## 6.7 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

## 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

ADVIA Centaur CP Immunoassay system

### 7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

### 7.3 Supplies

- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips
- Cuvettes
- Tips

## 8. PROCEDURE

**NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.**

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

<b>8.1</b>	<b>Loading samples into Sample Rack</b>
1.	Position the barcode label vertically on the sample tube approximately 2 cm (0.8 in) from the top.
2.	Move the tube-type selector on the rack to position B for short and C for long sample tubes.
3.	Place the sample tubes in the rack.
4.	Ensure that there is only one type of sample tube in the rack and that the tube-type selector is positioned correctly for that tube type. Ensure that the barcode labels are clearly visible above or between the slots in the rack.

<b>8.2</b>	<b>Loading Sample Racks into the System</b>
1.	Open the sample compartment door.
2.	Slide the sample rack into the appropriate lane. Use one continuous motion. The sample barcodes are read as the rack is inserted into the system. If the rack stops, or reverses, during its insertion, the barcodes may not be read.
3.	Ensure that the sample rack is inserted correctly, and locked into place. When the sample rack is inserted correctly, you will hear and feel it click into place.
4.	Close the sample compartment door.

<b>8.3</b>	<b>Scheduling Samples through the Sample Compartment Screen</b>
1.	At the workspace, select the sample compartment. Select the Sample Rack. Select a sample
2.	Select the sample type by toggling the sample selection button. The default is a routine patient sample ( <b>sm</b> ). If you want the sample to be processed before routine samples, select <b>stat</b> .
3.	Select the Sample ID field. The samples are numbered 1-12. Number 1 is the sample at the back of the sample compartment, and number 12 is the sample at the front of the sample compartment.
4.	If the sample ID was not entered using a barcode label on the sample tube, enter the sample ID and then press <b>Enter</b> .
5.	Select the assays or the profiles to process against the sample.
6.	Select <b>Close</b> .

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## 9. CALCULATIONS

The instrument automatically calculates the concentration of BNP in pg/mL.

## 10. REPORTING RESULTS AND REPEAT CRITERIA

### 10.1 Interpretation of Data

None required

### 10.2 Rounding

No rounding is necessary. Instrument reports results in whole numbers.

### 10.3 Units of Measure

pg/mL

### 10.4 Clinically Reportable Range (CRR)

2 - 10,000 pg/mL

### 10.5 Repeat Criteria and Resulting

IF the result is ...	THEN...
< 2 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.0 pg/mL
≥ 5000 pg/mL	<b>On Board Automated Dilution:</b> Results ≥ 5000 pg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 10,000 pg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: “> 10,000 -REP” Bring to the attention of your supervisor prior to releasing result.

## 11. EXPECTED VALUES

### 11.1 Reference Ranges

0 – 100 pg/mL

### 11.2 Critical Values

None established

### 11.3 Priority 3 Limit(s)

None established

## 12. CLINICAL SIGNIFICANCE

This assay is indicated for the measurement of plasma BNP as an aid in the diagnosis and assessment of the severity of heart failure. In patients with acute coronary syndromes (ACS), this test, in conjunction with other known risk factors, can also be used to predict survival as well as to predict the likelihood of future heart failure.

Heart failure is an important clinical syndrome which compromises left ventricular systolic or diastolic function or a combination of both. Heart failure occurs when the heart is unable to pump blood at a rate sufficient for metabolic requirements. Its most common causes are coronary artery disease, hypertension, valvular heart diseases and cardiomyopathies. Accurate and early diagnosis is important since effective therapeutic interventions (e.g., angiotensin converting enzyme inhibitors, beta-blockers) are available, which improve both morbidity and mortality. Based on clinical signs and symptoms, the severity of heart failure is classified into four classes of increasing disease progression according to the New York Heart Association classification.

## 13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/Cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report containing such error messages should be held for follow-up. Refer to you ADVIA Centaur CP Operator's Guide.

## 14. LIMITATIONS OF METHOD

### 14.1 Analytical Measurement Range (AMR)

2 - 5000 pg/mL

### 14.2 Precision

Six samples were assayed 4 times, in 20 runs, on 2 systems over a period of 20 days. The following results were obtained:

Mean pg/mL	Within-Run % CV	Run-to-Run % CV	Total CV %
29.4	4.3	1.9	4.7
48.5	2.5	2.1	3.5
410	1.8	1.9	2.8
458	2.0	1.5	2.8

1452	2.0	0.5	2.3
1736	2.1	1.7	2.9

**14.3 Interfering Substances**

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.

Specimens that are....	Demonstrate $\leq 5\%$ change in results up to...
Lipemic	800 mg/dL of triglycerides 1000 mg/dL of cholesterol
Uremic	200 mg/dL of urea 2.5 mg/dL of creatinine
Icteric	25 mg/mL of unconjugated bilirubin

**14.4 Clinical Sensitivity/Specificity/Predictive Values**

N/A

**15. SAFETY**

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

**16. RELATED DOCUMENTS**

1. ADVIA Centaur CP Operator's Manual
2. Laboratory Quality Control Program
3. QC Schedule for ADVIA Centaur CP
4. Laboratory Safety Manual
5. Material Safety Data Sheets (MSDS)
6. Quest Diagnostics Records Management Procedure
7. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
8. Hemolysis, Icteria and Lipemia; Interference from (Lab policy)
9. Repeat Testing Requirement (Lab policy)
10. Current Allowable Total Error Specifications at [http://questnet1.qdx.com/Business\\_Groups/Medical/qc/docs/qc\\_bpt\\_tea.xls](http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls)
11. Current package insert BNP Reagent Cartridge

**17. REFERENCES**

1. Liquichek™ Cardiac Markers Plus Control, Bio-Rad Laboratories revised 11/2011
2. Package Insert, BNP Calibrator, Siemens Diagnostics revised 07/2008

**18. REVISION HISTORY**

Version	Date	Section	Reason	Reviser	Approval

**19. ADDENDA**

None

Technical SOP

<b>Title</b>	<b>Intact Parathyroid Hormone (iPTH) by ADVIA Centaur CP</b>	
<b>Prepared by</b>	Ashkan Chini	Date: 5/1/2012
<b>Owner</b>	Jean Buss, Robert SanLuis	Date: 5/1/2012

<b>Laboratory Approval</b>		<b>Local Effective Date:</b>
Print Name and Title	Signature	Date
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**1. TEST INFORMATION**

<b>Assay</b>	<b>Method/Instrument</b>	<b>Local Code</b>
Intact Parathyroid Hormone	ADVIA Centaur CP	IOIPTH

<b>Synonyms/Abbreviations</b>
iPTH

<b>Department</b>
Chemistry

**2. ANALYTICAL PRINCIPLE**

The ADVIA Centaur iPTH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of an antihuman PTH antibody in the Lite Reagent and an antihuman PTH antibody in the Solid Phase Reagent. The first antibody is a polyclonal goat antihuman PTH (N-terminal 1-34) antibody labeled with acridinium ester. The second antibody is a biotinylated polyclonal goat antihuman PTH (39-84 region) antibody. Streptavidin in the Solid Phase is covalently coupled to paramagnetic latex particles.

**3. SPECIMEN REQUIREMENTS**

**3.1 Patient Preparation**

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

**3.2 Specimen Type & Handling**

Criteria	
Type -Preferred -Other Acceptable	Plasma (K2 EDTA) None
Collection Container	Lavender Top Tube
Volume - Optimum - Minimum	Full Tube 1 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 2-8° C 72 hours
	Frozen: -70° C N/A
Timing Considerations	Correct handling of patient samples is critical to ensure the integrity of the intact PTH molecule. Intact PTH has been demonstrated to be labile and is susceptible to fragmentation. This instability depends on both time and temperature.

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Criteria	
<b>Unacceptable Specimens &amp; Actions to Take</b>	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for “test not performed” message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
<b>Compromising Physical Characteristics</b>	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
<b>Other Considerations</b>	Centrifuge samples at $\geq 1000$ g for at least 15 minutes

#### 4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering “SAFETY” for additional information.

##### 4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
ADVIA Centaur iPTH Ready Pack	Siemens reagent cartridge Cat. No. 129501
ADVIA Centaur Multi-Diluent 11	Siemens reagent cartridge Cat. No. 117228
ADVIA Centaur R1 Acid Reagent	Siemens reagent Cat. No. 00497043
ADVIA Centaur R2 Base Reagent	Siemens reagent Cat. No. 00497043
ADVIA Centaur Wash 1 Solution	Siemens reagent Cat. No. 112351
ADVIA Centaur CSC	Siemens reagent Cat. No. 112748

##### 4.2 Reagent Preparation and Storage

**NOTES:** Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Assay Kit: ADVIA Centaur iPTH Ready Pack (primary reagent pack)	
<b>Reagent a</b>	Lite Reagent (Volume 5 mL)
<b>Reagent b</b>	Solid Phase (Volume 20 mL)
<b>Manufacturer’s Information</b>	Centaur CP iPTH Master Curve Card

<b>Storage</b>	Store the reagent upright at 2 - 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 28 days.
<b>Preparation</b>	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.

<b>Reagent</b>	<b>ADVIA Centaur Multi-Diluent 11</b> (ancillary reagent pack)
<b>Container</b>	Reagent Cartridge
<b>Storage</b>	Store the reagent up right at 2 - 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 28 days.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur R1 Acid Reagent &amp; R2 Base Reagent</b>
<b>Container</b>	Reagent bottle (300 mL)
<b>Storage</b>	Store at 2 - 25° C Use at 18 - 30° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 30 days. Avoid exposure to light.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur Wash 1 Solution</b>
<b>Container</b>	Reagent bottle (1500 mL)
<b>Storage</b>	Store at 2 - 25° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Onboard reagents are stable for 30 days.
<b>Preparation</b>	This reagent is liquid and ready to use.

<b>Reagent</b>	<b>ADVIA Centaur Cleaning Solution Concentrate</b>
<b>Container</b>	Reagent bottle (70 mL)
<b>Storage</b>	Store at 2 – 8° C
<b>Stability</b>	Reagent is stable until expiration date stamped on the pack label. Prepared reagent is stable for 7 days.
<b>Preparation</b>	<ol style="list-style-type: none"> <li>Carefully pour a container of ADVIA Centaur CP Cleaning Solution Concentrate into the cleaning bottle.</li> <li>Add enough reagent grade water to the bottle or container to bring the total volume of cleaning solution to 2 liters.</li> </ol>

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## 5. CALIBRATORS/STANDARDS

### 5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Low and High Calibrator	Siemens Intact PTH calibrator, Cat. No. 02698011

### 5.2 Calibrator Preparation and Storage

**NOTE:** Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Intact PTH Calibrator (Low & High)
Preparation	<ul style="list-style-type: none"> <li>Add 1.0 mL of reagent grade water into each calibrator vial.</li> <li>Let the calibrators stand for 30 minutes at room temperature (18 - 25° C) to allow the lyophilized material to dissolve.</li> <li>Gently mix the calibrators before using.</li> </ul>
Storage/Stability	<ul style="list-style-type: none"> <li>Store at <math>\leq -20^{\circ}</math> C</li> <li><b>Unopened Calibrator</b> is stable until the expiration date on the vial.</li> <li><b>Opened Calibrator:</b> once reconstituted the calibrator is stable for 60 days when stored at <math>\leq -20^{\circ}</math> C. <b>Freeze-thaw is recommended one time only after reconstitution.</b></li> <li><b>Opened Calibrator:</b> once reconstituted the calibrator is stable for 4 hours either onboard or at room temperature (18 - 25° C)</li> </ul>

### 5.3 Calibration Procedure

Criteria	Special Notations
Frequency	<ul style="list-style-type: none"> <li>When the calibration interval expires</li> <li>Calibration Interval is every 14 days</li> <li>A calibration is invalid</li> <li>New lot or shipment of assay reagents</li> <li>Controls are repeatedly out of range.</li> </ul>
Procedure	<ol style="list-style-type: none"> <li>Enter the calibrator information provided on the ADVIA Centaur CP side of the calibrator assignment value card. Ensure you use the ADVIA Centaur CP side of the card.</li> <li>Enter the Master Curve information from the cards provided with the primary reagent packs. Ensure you use the ADVIA Centaur CP side of the card.</li> </ol>

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	<ol style="list-style-type: none"><li>3. Load the low and high calibrators into appropriate sample pour-off tubes that accommodate the Siemens-supplied barcode label.</li><li>4. Attach the Siemens-supplied barcode labels to the pour off tubes.</li><li>5. Load the sample tubes into a sample rack. Move the tube-type selector on the rack to position A.</li><li>6. Load the rack in the sample compartment.</li><li>7. On the Sample Compartment screen, select the lane containing the sample rack with the calibration material.</li><li>8. Confirm or enter the calibrator information.</li><li>9. Select <b>Close</b> to return to the workspace.</li><li>10. At the workspace, select the primary reagent area.</li><li>11. Select the assay to calibrate.</li><li>12. Select <b>Calibrate</b>. The system automatically begins sampling the calibrator material.</li></ol> <p><b>NOTE:</b> The Calibrate button is not active on the Primary Reagent Screen in the following situations:</p> <ul style="list-style-type: none"><li>• there are no calibrators defined</li><li>• there are no Master Curves defined</li><li>• there is no reagent onboard</li><li>• the calibration material for defined lots is expired</li><li>• there is no calibrator material in the sample compartment</li></ul> <ol style="list-style-type: none"><li>13. Select <b>Close</b> to return to the workspace.</li><li>14. To check the time due for the calibration:<ol style="list-style-type: none"><li>a. At the workspace, select <b>Results</b>.</li><li>b. Select the Calibrations tab to view the Time Due stamp for the assay you are calibrating.</li><li>c. Select <b>Close</b>.</li></ol></li></ol>
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### 5.5 Tolerance Limits

IF.....	THEN.....
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

## 6. QUALITY CONTROL

### 6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek Specialty Immunoassay Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat. No. 364, 365 and 366

### 6.2 Control Preparation and Storage

**NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.**

<b>Control</b>	Liquichek Specialty Immunoassay Control Levels 1, 2 and 3
<b>Preparation</b>	<ul style="list-style-type: none"> <li>Allow the frozen control to stand at room temperature (18 - 25°C) until it is completely thawed.</li> <li>Before sampling, gently swirl the vial several times to ensure homogeneity.</li> <li>Promptly replace the stopper and return to 2-8° C storage after each use.</li> </ul>
<b>Storage/Stability</b>	<ul style="list-style-type: none"> <li>Store at ≤ -20° C</li> <li><b>Unopened Quality Control</b> is stable until the expiration date on the vial.</li> <li><b>Opened Quality Control:</b> Once the product is thawed and opened, Intact PTH will be stable for 23 days when stored tightly capped at 2-8° C.</li> </ul>

### 6.3 Frequency

Analyze all 3 Levels of QC material after each calibration. Each day of patient testing, Levels 1 and 3 are run prior to patient samples. Level 2 QC is run at conclusion of patient testing for the day.

### 6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the Laboratory Information System (LIS), and may be posted near the instrument for use during computer downtime.
2	<b>Run Rejection Criteria</b> <ul style="list-style-type: none"> <li>Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported.</li> <li>The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.</li> </ul>
3	<b>Corrective Action:</b> <ul style="list-style-type: none"> <li>All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be <u>reanalyzed according to the Laboratory QC Program</u>. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program.</li> <li>Corrective action documentation must follow the Laboratory Quality Control Program.</li> </ul>
4	<b>Review of QC</b> <ul style="list-style-type: none"> <li>QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee.</li> <li>If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.</li> </ul>

IF the Quality Control ...	THEN...
does not fall within the Expected Values	<ul style="list-style-type: none"> <li>Verify that the materials are not expired.</li> <li>Verify that required maintenance was performed.</li> <li>Verify that the assay was performed according to the instructions for use.</li> <li>Rerun the assay with fresh quality control samples.</li> <li>If necessary recalibrate the assay and repeat quality control</li> </ul>

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## **6.5 Review Patient Data**

Technologist must review each result print-out for error messages. Refer to the Centaur Operator's Guide for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). Resolve any problems noted before issuing patient reports.

## **6.6 Documentation**

- QC tolerance limits are programmed into the instrument and the LIS. The LIS calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

## **6.7 Quality Assurance Program**

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

## **7. EQUIPMENT and SUPPLIES**

### **7.1 Assay Platform**

ADVIA Centaur CP Immunoassay system

### **7.2 Equipment**

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

### 7.3 Supplies

- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips
- Cuvettes
- Tips

## 8. PROCEDURE

**NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.**

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

8.1	Loading samples into Sample Rack
1.	Position the barcode label vertically on the sample tube approximately 2 cm (0.8 in) from the top.
2.	Move the tube-type selector on the rack to position B for short and C for long sample tubes.
3.	Place the sample tubes in the rack.
4.	Ensure that there is only one type of sample tube in the rack and that the tube-type selector is positioned correctly for that tube type. Ensure that the barcode labels are clearly visible above or between the slots in the rack.

8.2	Loading Sample Racks into the System
1.	Open the sample compartment door.
2.	Slide the sample rack into the appropriate lane. Use one continuous motion. The sample barcodes are read as the rack is inserted into the system. If the rack stops, or reverses, during its insertion, the barcodes may not be read.
3.	Ensure that the sample rack is inserted correctly, and locked into place. When the sample rack is inserted correctly, you will hear and feel it click into place.
4.	Close the sample compartment door.

8.3	Scheduling Samples through the Sample Compartment Screen
1.	At the workspace, select the sample compartment. Select the Sample Rack. Select a sample
2.	Select the sample type by toggling the sample selection button. The default is a routine patient sample (smp). If you want the sample to be processed before routine samples, select stat.
3.	Select the Sample ID field. The samples are numbered 1-12. Number 1 is the sample at the back of the sample compartment, and number 12 is the sample at the front of the sample compartment.

<b>8.3</b>	<b>Scheduling Samples through the Sample Compartment Screen</b>
4.	If the sample ID was not entered using a barcode label on the sample tube, enter the sample ID and then press <b>Enter</b> .
5.	Select the assays or the profiles to process against the sample.
6.	Select <b>Close</b> .

**9. CALCULATIONS**

The instrument automatically calculates the concentration of iPTH in pg/mL.

**10. REPORTING RESULTS AND REPEAT CRITERIA**

**10.1 Interpretation of Data**

None required

**10.2 Rounding**

No rounding is necessary. Instrument reports results up to one decimal point.

**10.3 Units of Measure**

pg/mL

**10.4 Clinically Reportable Range (CRR)**

2.5 – 9,500.0 pg/mL

**10.5 Repeat Criteria and Resulting**

IF the result is ...	THEN...
< 2.5 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.5 pg/mL
≥ 1900.0 pg/mL	<b>On Board Automated Dilution:</b> Results ≥ 1900.0 pg/mL will automatically have repeat testing performed into the instrument using dilution factor of 5. No multiplication is necessary.
> 9,500.0 pg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: “> 9,500.0 -REP” Bring to the attention of your supervisor prior to releasing result.

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## 11. EXPECTED VALUES

### 11.1 Reference Ranges

11.0 – 80.0 pg/mL

### 11.2 Critical Values

None established

### 11.3 Priority 3 Limit(s)

None established

## 12. CLINICAL SIGNIFICANCE

This assay is intended to be used to aid in the differential diagnosis of hyperparathyroidism, hypoparathyroidism, or hypercalcemia of malignancy.

Parathyroid hormone (PTH), produced by the parathyroid gland, is the major circulating factor regulating extracellular calcium concentration. Abnormally low-ionized calcium concentrations trigger the secretion of PTH. The PTH molecules bind to type 1 parathyroid hormone receptors in target tissues and initiate a sequence of reactions resulting in increased extracellular calcium concentrations. PTH stimulates osteoclastic bone resorption resulting in the release of calcium from bone. PTH stimulates transcellular calcium reabsorption from the renal tubules and stimulates the kidney to produce 1,25-dihydroxyvitamin D which acts on the intestines to increase calcium reabsorption. In most clinical conditions, rising levels of extracellular calcium suppresses PTH secretion through a negative feedback mechanism.

Parathyroid hormone increases the rate of bone metabolism. Depending on the age of the patient, the bones involved, and the concentrations of the hormone in circulation over time, the effect on the bone can be either catabolic or anabolic. Consistently high concentrations of parathyroid hormone generally have a catabolic effect and intermittent, slightly elevated concentrations have an anabolic effect.

## 13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/Cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report containing such error messages should be held for follow-up. Refer to you ADVIA Centaur CP Operator's Guide.

## 14. LIMITATIONS OF METHOD

### 14.1 Analytical Measurement Range (AMR)

2.5 – 1900.0 pg/mL

### 14.2 Precision

Three samples were assayed 12 times, in each of 12 runs, on 3 systems over a period of 4 days. The following results were obtained:

Mean pg/mL	Within-Run % CV	Run-to-Run % CV	Total CV %
40.4	5.2	5.8	7.8
223.8	3.4	1.5	7.0
859.3	3.5	2.8	4.6

### 14.3 Interfering Substances

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.

Specimens that are....	Demonstrate $\leq 10$ % change in results up to...
Hemolyzed	200 mg/dL of hemoglobin
Lipemic	3000 mg/dL of triglycerides
Icteric	40 mg/dL of bilirubin
Biotin	1000 ng/mL of biotin

### 14.4 Clinical Sensitivity/Specificity/Predictive Values

The cross-reactivity of the ADVIA Centaur Intact PTH assay was determined by spiking samples with the PTH fragments and compounds listed below at the indicated levels. There was no significant effect on the intact PTH measurement.

Cross-reactant	Amount added (pg/mL)	% Cross-reactivity
PTH 1 - 34 fragment	300	0.74
PTH 39 – 68 fragment	100,000	0.005
PTH 39 – 84 fragemnt	100,000	0.024
PTH 44 – 68 fragment	100,000	0.007
PTH 53 – 84 fragment	100,000	0.003
Calcitonin	100,000	0.0004

## 15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

## 16. RELATED DOCUMENTS

1. ADVIA Centaur CP Operator's Manual
2. Laboratory Quality Control Program
3. QC Schedule for ADVIA Centaur CP
4. Laboratory Safety Manual
5. Material Safety Data Sheets (MSDS)
6. Quest Diagnostics Records Management Procedure
7. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
8. Hemolysis, Icteria and Lipemia; Interference from (Lab policy)
9. Repeat Testing Requirement (Lab policy)
10. Current Allowable Total Error Specifications at [http://questnet1.qdx.com/Business\\_Groups/Medical/qc/docs/qc\\_bpt\\_tea.xls](http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls)
11. Current package insert Intact PTH Reagent Cartridge

## 17. REFERENCES

1. Package Insert, Intact PTH Quality Control 1,2,3, Siemens Diagnostics revised 2011
2. Package Insert, Intact PTH Calibrator, Siemens Diagnostics revised 2011
3. Package Insert, Intact PTH reagent pack, Siemens Diagnostics revised 2011

**18. REVISION HISTORY**

Version	Date	Section	Reason	Reviser	Approval

**19. ADDENDA**

None