|  |  |  |  |
| --- | --- | --- | --- |
|  | **Anti-CCP, IgG****(aCCP)****CL-CH183** | **Dept:** | Clinical Core Lab-Chemistry Section |
| **Effective Date:** | 8/28/2019 |
| **Revised Date:** |  |
| **Contact:** | Clinical Core Lab-Chemistry Management |
| **Name & Title:** Gregory J. Pomper, MD  Medical Director of Pathology Laboratories | **Date:** |  |
| **Signature:** |

1. **General Procedure Statement:**
	1. **Scope:** To provide laboratory testing personnel with instructions for performing

laboratory procedures as deemed appropriate by industry practices and regulatory agencies to assist in quality patient care.

* 1. **Responsible Department/Party/Parties:**
		1. Procedure owner: Clinical Core Laboratory Management-Chemistry
		2. Procedure: Clinical Core Laboratory Personnel
		3. Procedure prepared by: Greg Horton
		4. Supervision: Clinical Core Laboratory Management-Chemistry

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Chemistry

1. Implementation: Clinical Core Laboratory Management-Chemistry

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Chemistry

1. **Definitions:**
2. **Procedure:**

**Anti-CCP IgG (aCCP)**

**Analyzer : ADVIA Centaur XP**

|  |
| --- |
| **Principles of the Procedure** |
| The ADVIA Centaur aCCP assay is a fully automated, two-step immunoassay using chemiluminescent technology. The assay utilizes an acridinium ester-labeled anti-human IgG as the Lite Reagent. The Solid Phase consists of biotinylated CCP coupled to streptavidin which is then coated onto magnetic latex microparticles. |
| **Intended Use** |
| The ADVIA Centaur® anti-CCP IgG (aCCP) assay is for *in vitro* diagnostic use in the semiquantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma (K2-EDTA and lithium heparin) using the ADVIA Centaur XP and ADVIA Centaur XPT systems. Detection of anti-CCP antibodies is used as an aid in the diagnosis of Rheumatoid Arthritis (RA) and should be used in conjunction with other clinical information. Autoantibody levels represent one parameter in a multi-criteria diagnostic process, encompassing both clinical and laboratory-based assessments. |
| **Specimen Types** |
| Human Serum, Plasma (K2-EDTA, lithium-heparin). **SERUM is the specimen type used in this laboratory.** |
| **Specimen Stability** |
| Handle all specimens as if capable of transmitting disease.Follow the instructions provided with your specimen collection device for use and processing.Separated specimens are stable for up to 22 hours at room temperature, 7 days at 2–8°C. If testing will be delayed more than 7 days, store specimens at -20°C or colder. Avoid more than 2 freeze/thaw cycles. Do not store in a frost-free freezer.Thoroughly mix all thawed samples and centrifuge before using. Thawed frozen specimens which are turbid must be clarified by centrifugation prior to testing. |
| **Minimum Sample Volume** |
| 10 µLAdditional instructions for patient preparation as designated by this laboratory: NoneAdditional specimen type information: Serum is used in this laboratory.Additional handling conditions as designated by this laboratory:All specimens are labeled with a unique barcode identifier. Specimens received in a standard 13x75 vacutainer tube are processed by the Beckman Power Processor and delivered to attached instruments robotically. Those samples which are of insufficient volume or are of any tube type other than a 13x75 vacutainer are centrifuged and processed manually |
| **Preparing the Reagents** |
| All reagents are liquid and ready to use. Remove all of the reagents from the refrigerator, and mix all primary reagent packs by hand. Visually inspect the bottom of the reagent packs to ensure that all particles are dispersed and resuspended before loading them onto the system. |
| **Storage and Stability** |
|  |
| Store ADVIA Centaur aCCP reagent packs upright at 2–8°C away from heat and light sources. Reagent packs loaded on the system are protected from light. Reagents are stable at 2–8°C until the expiration date on the product.Do not use ADVIA Centaur materials beyond the expiration date printed on the product. The ADVIA Centaur aCCP assay reagents are stable unopened until the expiration date on the product or onboard the system for 60 days. Discard reagent packs at the end of the 60-day onboard stability interval. Do not use reagents beyond the expiration date.Location of reagents within this laboratory:Refrigerator #14 **Loading Reagents**You can monitor the volumes of primary and ancillary reagents using the Worklist – Reagent Inventory window.**CAUTION:** Mix all reagent packs by hand before loading them onto the system. It is important to minimize foaming. For detailed information about preparing reagents for use, refer to the *Operator's Guide*.***Loading Primary Reagents***1. Mix the primary reagent pack by hand. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended.
2. Open the primary reagent door.
3. Identify a location for the pack by using the arrows as a placement guide.
4. Pull the reagent holder towards you.
5. Load the pack on the reagent holder.
6. Press down on the top of the pack to ensure it is correctly seated on the reagent holder and then push the reagent holder back firmly until you hear the pack lock in place.
7. Close the primary reagent door.
8. Press the sample start button for the system barcode scanner to rescan the reagent barcodes and acknowledge any reagent changes.

***Loading Ancillary Reagents***1. Mix the ancillary reagent pack by hand.
2. Load the pack in the ancillary reagent entry with the barcoded side facing outward.

An LED next to the ancillary reagent entry turns green to indicate that the pack is loading.**Calibrator Material**Siemens aCCP calibratorSiemens aCCP calibrator is inside the reagent kit. See Storage and Stability section.Location of calibrator within this laboratory:Refrigerator #14 **Defining the Master Curve**The ADVIA Centaur aCCP assay requires a Master Curve calibration when using a new lot number of Binary Lite Reagent and Solid Phase. For each new lot number of Binary Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.1. At the workspace, select **Calibration**.
2. Select **Master Curve Definition**.
3. Select **Scan Data**.
4. Use the handheld barcode reader to scan the barcodes on the *Master Curve* card.

**IMPORTANT:** Ensure you are scanning the side of the *Master Curve* card labeled ADVIA Centaur.**NOTE:** The table on the Master Curve card represents the fields as they are displayed in the ADVIA Centaur System only. Use the illustrations in the *Using the Master Curve Card and Calibration – Master Curve Definition Window Customer Bulletin*, part number 078D0560-01 Rev. A, to match the fields in the *Master Curve* card with the fields as they are displayed in the ADVIA Centaur XP System.1. Select **Save**.
 |
|  **Calibration**  Calibrate an assay in the following instances:* the calibration interval expires (use the Calibration – Summary window to view calibration status)
* a calibration is invalid
* a new lot of assay reagents is used
* controls are repeatedly out of range
1. Enter the calibrator information provided on the ADVIA Centaur *Calibrator Assigned Value* card.
2. Enter the Master Curve information from the *Master Curve* card provided with the primary reagent packs.
3. Load the reagent lot you want to calibrate.
4. At the workspace, select **Worklist**.
5. Select **Schedule**.
6. Select **Calibrator**.
7. Select **Schedule by SID** or **Schedule by Rack ID** to indicate how you want the system to identify the calibrator.
8. Select or enter a test. The calibrators previously defined for the test are automatically displayed.

**NOTE:** Calibrators do not display in the following situations:• there are no calibrators defined• there are no Master Curves defined• there is no reagent onboard• the calibration material for defined lots is expired1. Select a calibrator.
2. Select a reagent lot.
3. If you schedule by rack, enter the Rack ID in Rack.
4. Select **Save**.
5. Repeat steps 7 through 12 to schedule additional tests for a calibrator.
6. Label two sample cups with calibrator barcode labels: one for the low and another for the high.
7. Gently mix the calibrators and dispense at least 500 L of Low and High Calibrator into the labeled sample cups. The sample cups are marked at 1.0 mL intervals to assist in filling.
 |
| **Quality Control Material**anti-CCP quality controlSiemens aCCP Quality Control kit, ref# 10699150, 1 vial of Negative control, 1 vial of Positive controlLIS Codes: CCPN, CCPPLocation of control within this laboratory:Refrigerator #14 Controls are supplied liquid, ready to use. Stable until the expiration date on product at 2-8°C when unopened. Once opened, 90 days at 2-8°C.QC FrequencyAnalyze all levels of QC on each day that samples are analyzed.Analyze all levels of QC each time a calibration is performed. **Scheduling Controls*** + - 1. At the workspace, select **Worklist**.
			2. Select **Schedule**.
			3. Select **Control**.
			4. Select the assay.
			5. Select the correct lot# of control material

Select **Save****Troubleshooting Out-of-Range QC Values**If the quality control results do not fall within the Expected Values or within the laboratory’s established values, do not report results. Take the following actions.* Verify that the materials are not expired.
* Verify that required maintenance was performed.
* Verify that the assay was performed according to the instructions for use.
* Rerun the assay with fresh quality control samples.

If necessary, contact your local technical support provider or distributor for assistance.**Loading Sample Racks****NOTE:** You can also load samples using your laboratory automation system (LAS).

|  |  |  |
| --- | --- | --- |
|  |  | **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. |

1. If you use sample cups, label them with the appropriate barcode labels and dispense the sample into the labeled sample cups.
2. Move the tube-type selector to the position for the tube type you are loading in the rack or for multiple tube types.

**NOTE:** When you use an ADVIA Centaur rack, you can only load one tube type in the rack.1. Place the sample tubes or cups in the rack.

**CAUTION:** Do not load capped sample tubes. Ensure that all caps are removed from the sample tubes before loading the rack onto the system. Leaving the caps on the tubes can cause mechanical damage to the system.1. Ensure that the barcode labels are clearly visible above or between the slots in the rack.
2. Load the rack in the sample entry queue. Ensure that the notch in the rack rests on the raised area of the sample entry queue.

**CAUTION:** Do not manually push a rack into the inprocess queue and do not manually place sample racks near the entrance to the inprocess queue without using the raised area of the sample entry queue as a positioning guide. **CAUTION:** Do not press the sample start button until you close the primary reagent door and ensure that all reagent barcodes were read correctly.**F. Loading STAT Samples**1. STAT samples can be placed in a rack and loaded in the Stat entry. The system assigns STAT priority to these samples and moves the rack to the inprocess queue as soon as space is available.
2. Samples can also be scheduled as STAT samples at the LIS or at the Worklist – Schedule window and loaded in the sample entry queue. STAT samples loaded in the sample entry queue are processed as soon as the system scans the barcode label and loads the sample rack into the inprocess queue.
 |
|  Analytic Process**Instrument Operation and System Description**The ADVIA Centaur and ADVIA Centaur XP systems are automated, direct chemiluminescent immunoassay analyzers that offer optimal productivity and efficiency. No-pause reloading of reagents, samples, and supplies means that the system is always ready to process samples.Sample racks are loaded in the sample entry queue and the sample start button is pressed to activate the test sequence. The sample entry queue moves the sample racks to the inprocess queue, where the sample is aspirated and dispensed it into a cuvette in the incubation ring. Reagents are dispensed into the cuvette, the reaction mixture is incubated, and then the cuvette is moved to the wash station where the magnetic particles are washed. Acid Reagent is dispensed into the cuvette and then the cuvette is moved into the luminometer. The addition of Base Reagent causes the chemiluminescent reaction to occur. The PMT measures the chemical light reaction that takes place.**A. Processing Start and Fast Stop** If the sample entry queue is in standby, press the sample start button to activate the sample entry queue. The sample entry queue moves the sample racks one at a time to the inprocess queue.There are two fast stop buttons on the system. Use these buttons if there is an obstruction that could cause serious system or physical damage.**B. Start-up**1. Start the system (if required) and sign in.
2. Load required reagents and consumables.
3. Schedule (if needed) and load samples and controls.
4. Press the sample start button.

**C. Managing Supplies** While the system is running, you can manage the following supplies without interrupting the run.

|  |  |
| --- | --- |
| * Primary reagents
 | * Tips
 |
| * Ancillary reagents
 | * Cuvette waste
 |
| * System fluids
 | * Tip tray waste
 |
| * Water
 | * Tip waste
 |
| * Cuvettes
 | * Liquid waste
 |

 |

**D. Scheduling a Run/Entering a Worklist**

You can schedule a run through a laboratory information system (LIS) or you can manually create a worklist. For detailed information about scheduling a run, refer to the ADVIA Centaur and ADVIA Centaur XP systems operating instructions.

**LIS Supplied Worklist**

The system can obtain worklist entries from an LIS by manually requesting the worklist (Receive Requests from LIS) or by allowing the system to automatically request worklist entries when a sample is added to the inprocess queue (System Automatically Queries Host for Worklist*).*

**Manual (Operator-Initiated) Worklist**

**Scheduling Tests for an Individual Patient Specimen**

1. At the workspace, select **Worklist**.
2. Select **Schedule**.
3. Select **Patient**.
4. Select **Schedule by SID** or **Schedule by Rack ID** to indicate how you want the system to identify the sample.
5. Enter the SID or the Rack ID and then press **Enter.** Alternatively, select **Scan Data** to scan the SID using the hand-held barcode reader.
6. If you want to process the sample before routine samples, select **Stat**.
7. Select or enter the tests or the profiles for the sample.
8. Optionally, select **Demographics**, enter the demographic information, and select **Continue**.
9. Select **Save**.

**Interferences**

|  |
| --- |
| Potential interference in the ADVIA Centaur aCCP assay is designed to be < 10%. Interfering substances at the levels indicated in the table below were tested as described in CLSI Document EP7-A2 using the ADVIA Centaur aCCP assay.Two levels of anti-CCP IgG were tested with each of the following substances at the levels indicated, and caused no significant interference in the ADVIA Centaur aCCP assay.Assay results obtained at individual laboratories may vary from the data presented.**High-Dose Hook Effect**Patient samples with high anti-CCP IgG levels can cause a paradoxical decrease in the Relative Light Units (RLUs) (high-dose hook effect). In this assay, patient samples with anti-CCP IgG levels as high as 3000.00 U/mL will assay greater than 200.00 U/mL.**Specificity**Cross-reactivity was tested in the presence and absence of anti-CCP IgG according to CLSI EP7-A2 using the ADVIA Centaur aCCP assay. Populations evaluated in the study included Sm, RNP, SSB, Scl-70, Jo-1, Ribosomal P, M2, TPO, ds-DNA, SSA and Chromatin. The study showed no clinically significant cross-reactivity of the CCP antigen with any of these auto-antibodies.**Other**Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference, which can potentially cause an anomalous result.Due to the specific characteristics of antigen/antibody interactions it is not the concentration of antibody which is determined, but the activity. Since patient sera contain heterogeneous antibody populations, some samples may exhibit non-linear dilution, especially at high antibody levels. |
| **Interpretation of Results** |
| The system reports the concentration of anti-CCP IgG in U/mL.Do not use ADVIA Centaur aCCP test results interchangeably with test results from other anti- CCP IgG assays.Sample results are invalid and must be repeated if the controls are out of range.The cut-off value was determined by a Receiver Operating Characteristic (ROC) analysis with a balanced consideration of sensitivity and specificity according the CLSI EP24-A2. Using the recommended clinical cut-off value of 5.00 U/mL: * a result > 5.00 U/mL indicates that anti-CCP IgG antibodies were detected in the sample.
* a < 5.00 U/mL indicates that anti-CCP IgG antibodies were not detected in the sample.

Results of this assay should always be interpreted in conjunction with patient’s medical history, clinical presentation and other findings. |
| **Analytical Measuring Range** |
| 0.40–200.00 U/mL |
| **Dilutions** |
| The following information pertains to dilutions:Manually dilute and retest patient samples with levels of anti-CCP IgG greater than 200.00 U/mL to obtain accurate results.This assay has been validated for use with a dilution factor x 10 (1 part sample plus 9 parts diluent) for a range up to 2000.00 U/mL. Results below the measuring range are reported as less than 0.40 U/mL.Use Multi-Diluent 1 to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result. |

|  |
| --- |
| **Sensitivity** |
| **Clinical Specificity**The specificity assessment of the ADVIA Centaur aCCP assay was conducted on 22 subgroups of non-RA subjects (n = 460) with potentially cross-reacting conditions and disease states where other auto-antibodies may be present in the subject samples. The following disease states were evaluated:**Detection Capability**The ADVIA Centaur aCCP assay is designed to have a Limit of Detection (LoD) of less than 1.50 U/mL. The LoD was determined as described in CLSI Document EP17-A2.The LoD is defined as the lowest concentration of anti-CCP IgG that can be detected with greater than or equal to 95% probability. The LoD was determined to be 0.40 U/mL.  |
| **Expected Values** |
| * a result > 5.00 U/mL indicates that anti-CCP IgG antibodies were detected in the sample.
* a result < 5.00 U/mL indicates that anti-CCP IgG antibodies were not detected in the sample
 |
|  |
| **Critical Values** |
| No established critical value in this laboratory |
| **Reporting** |
| Results are auto validated and transmitted from the Centaur XP to the LIS, Beaker. Values < 5.00 U/mL are reported as “< 5.00 U/mL”. Values from 5.00 to 200.00 are reported as a numeric value. Specimens reading >200.00 U/mL are reported as “ >200.00 U/mL”. Dilutions may be performed if requested following the dilution protocol outlined in this procedure. |

**Note** Appendices L1 and L3 of CLSI QMS02-A6, published 2/28/2013, guided the creation of this document.

Siemens document number: RPBL1202R4D\_aCCP\_XPT\_US ONLY\_EN

1. **Review/Revision/Implementation:**
	1. Review Cycle: 2 years
	2. Office of Record: Department of Clinical Core Laboratory-Chemistry
	3. All new procedures and procedures that have major revisions must be signed by the Laboratory Director.
	4. All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.
2. **Related Procedures:**
3. **References, National Professional Organizations, etc.:**

Siemens ADVIA Centaur XP and ADVIA Centaur XPT aCCP Technical Procedure RPBL1202R4D\_aCCP\_XPT\_US ONLY\_EN

Siemens ADVIA Centaur QC aCCP Quality Control Insert RPBL1133/R3, Rev. C, 2016-08

Siemens ADVIA Centaur XP Operator’s Guide 078D1064-01 Rev. B

1. **Attachments:**
2. **Revision Dates:**

|  |  |  |
| --- | --- | --- |
| **Review Date** | **Revision Date** | **Signature** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |