Heparin Xa CS 2500 & Ca 660

INTENDED USE

In-vitro diagnostic automated chromogenic assay for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity in human plasma collected from venous blood samples in 3.2 % sodium citrate tubes on the SYSMEX CS-2500 System and the SYSMEX CA-660 System in the clinical laboratory. For use with plasma from patients undergoing heparin anticoagulant therapy with either UFH or LMWH.

The performance of this device has not been established in neonate and pediatric patient populations.

SUMMARY AND EXPLANATION

Heparin (UFH and LMWH) considerably accelerates the inactivation of thrombin and coagulation factor Xa (Xa) by antithrombin (AT). For this reason UFH and LMWH preparations are widely used as prophylactic and therapeutic anticoagulants. The main clinical indications for UFH are in prevention and treatment of venous thromboembolism, in certain types of coronary artery syndrome and in thrombotic stroke. LMWH has been replacing UFH in many of the latter’s traditional indications. Due to numerous influences the anticoagulant effect of an identical dose of heparin varies from patient to patient.

Using the INNOVANCE HEPARIN assay it is possible to quantify the activity of UFH and LMWH in a patient's plasma and to verify the intended target level.

PRINCIPLE

The INNOVANCE HEPARIN assay is a one stage chromogenic assay. The reagent kit consists of two components. One component (Reagent) contains Xa, the other (Substrate) a chromogenic substrate specific for Xa. Upon mixing of Reagent and Substrate, Xa converts the chromogenic substrate into two products, one of them is paranitroaniline. The formation of paranitroaniline can be quantified by the coagulation analyzer employing light absorption at a specific wavelength (405 nm).

In the presence of a heparin containing sample the formation of paranitroaniline will be reduced in a time dependent manner. This is due to inhibition of Xa by the heparin/ATcomplex. This complex is formed in the patient's plasma and competes with the substrate conversion by Xa. The concentration of the complex is not only dependent on the concentration of heparin but also on the availability of the patient’s endogenous antithrombin. By comparison to a reference curve the heparin activity of the sample can be quantified.

To reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran sulfate is included in the reaction mixture.

SPECIMEN REQUIREMENTS

- 9:1 ration of whole blood to 3.2% sodium citrate anticoagulant should be collected.
- Room temperature

- The whole blood is centrifuged within 1 hour to achieve platelet poor plasma and tested within 4 hours from the time of specimen collection.
- Plasma can be frozen in an aliquot tube at -18 C or below for up to 3 days.
- Hemolysis may affect test results.
- Icteric or turbid samples must be rejected.

REAGENTS

- INNOVANCE Substrate Reagent:
    - Substrate reagent is ready to use.
    - Stabile for 8 weeks if kept refrigerated (2-8C) after opening.
    - Reagents muse be re-capped and placed back in the refrigerator if taken off the analyzer.
    - On board stability is 72 hours with the use of evaporation reagent caps on the reagent table.
- INNOVANCE Heparin Reagent:
    - Heparin Reagent is ready to use.
    - Stabile for 8 weeks if kept refrigerated (2-8C) after opening.
    - Reagents must be re-capped and placed back in the refrigerator if taken off the analyzer.

    - On board stability is 72 hours with the use of evaporation reagent caps on the reagent table.
- OVB:
    - OVB is ready to use.
    - Stabile for 8 weeks after opening if kept refrigerated (2-8C).
    - Reagents muse be re-capped and placed back in the refrigerator if taken off the analyzer.
    - On board stability is 8 hours on the buffer tray in a sample cup.
- Ca Clean I & II
    - Clean is ready to use.
    - Stabile for 2 weeks after opening.
    - Ca Clean I is to be kept in the refrigerator. It muse be re-capped and placed back in the refrigerator if taken off the analyzer.
    - Ca Clean II is kept at room temperature
    - On board stability is 120 hours on the reagent table.
- Controls-INNOVANCE Heparin Unfractionated and INNOVANCE Low Molecular Weight:
    - Reconstitute with labeled amount of DI H20.
    - Mix carefully to dissolve and allow to stand at room temperature for at least 15 minutes.

- Mix gently once more before use.
- After reconstitution controls can be stored at 2 - 8°C for 48 hours or at <-18°C for 4 weeks;
- Only use 200 uL of control and place in the SLD minicup in a rack.

CALIBRATION

INNOVANCE Heparin Calibrator (UFH and LMWH) is required for calibration. Refer to the CS-2500 Operator's Manual for instructions

Stability

15 - 25°C (24 hours) or 2 - 8°C (48 hours)

QUALITY CONTROL

INNOVANCE Heparin UF/LMW Controls 1 and 2 are used to validate the test performance. Controls should be run before running patient samples and must be repeated every 8 hours of patient testing.

There are two different orders in the hospital information system. Patients on unfractionated heparin will have an order (Heparin Anti-Xa Unfractionated). Run Heparin UF Controls 1 and 2. Patients on Low Molecular Weight Heparin (Lovenox) will have an order (Heparin Anti-Xa Low Molecular Weight). Run Heparin LMW Contols 1 and 2.

CONTROL PROCESSING

CS2500

1. QC will be run in a specimen rack, Refer to Sysmex CS 2500 Quality control procedure Sysmex CS-2500 Quality Control

- QC07 (Heparin UF Control 1)
- QC08 (Heparin UF Control 2)
- QC09 (Heaprin LMW Control 1)
- QC10 (Heparin LMW Control 2)

2. Select the test to be performed.
3. Press Start.

CA660 - Unfractionated Heparin Only

1. Refer to Sysmex CA-660 Quality Control Sysmex CA-660 Quality Control
2. Program the worklist by pressing "ID NO Entry"
3. Type the appropriate QC material ID
    - QC05 (Level 1 - Unfractionated Heparin)
    - QC06 (Level 2 - Unfractionated Heparin)
4. Select the test to Run (Heparin)
5. Open sample drawer and load the rack onthe instrument
6. Close sample drawer
7. Press Start
8. Indicate where the instrument should start in terms of reactions vessels
    - "Continue" will continue to work throught the rack of reaction vessels from the last spot used.

- "First Tube" the instrument begins in the upper right most position of the right reaction vessel rack.
    - NOTE: the will be your only option after shutting off the instrument.

PROCEDURE

CS2500

1. Automatic Run
    a. Place the capped barcoded samples in a tube rack with the barcodes facing the analyzer. Then place the rack into the right rack pool
    b. Press the start button on the instrument or alternatively, hit the start botton in the software
2. Manual Run
    a. From the Main Menu, press [ORDER].
    b. Press [SWITCH ORDER] and select [RACK ORDER].
    c. Enter the Rack No.
    d. Barcoded capped tube: place the barcoded sample tubes into the tube rack with the barcode facing the analyzer. Highlight the rack position for the specimen, press the test keys to select the tests to be run. Place the capped barcoded sample tubes on the right rack pool.
        1. If you wish to order a micro sampler (Mc), press the Mc parameter.

        i. NOTE: Micro sample refers to the instrument only sampling the amount for each test needed. No reflex resting will occur in micro sample mode.

    e. If the sample is to be run in a cup: Place the filled, labeled sample cup on a sample cup rack. Click "order Entry" on the menu to the right. Click on sample ID and either hand enter or scan the barcode using the barcode reader. Select the tests to be run. Press "OK"

    f. Press Start.

    g. Results will automatically transmit to Laboratory Information System.

CA 660 - Unfractional Heparin ONLY

1. Automatic Run

    a. Open specimen drawer and verify tht a rack is loaded.

    b. Place the uncapped barcoded sample tubes into the tube rack with the barcodes facing outward from the analyzer.

    c. Press Start

    d. Indicate if you wish the instrument to start at the first tube (this is the only option after the machine has been turned off) or continue through the reaction tube rack from the previous spot.

    e. The analyzer will read the barcode, aliquot the sample, and perform ordered tests.

1. When the instrument is done sampling the specimen, you will be allowed to open the sample drawer and add specimens when the instrument displays "Replace Rack? Yes"

2. Manual Run

    a. From the Main Menu, verify that a new worklist position is visible on the screen.

    b. Barcoded, uncapped tube: Place the barcoded sample tubes into the tube rack with the barcodes facing outward from the analyzer. With the cursor in the desired tube position in the worklist press the parameter keys to select the tests to be run. The instrument will apply the barcode to the orders in worklist. If more than one sample is to be programmed, use the down arrow and repeat above steps for each additional specimens.

    c. If the sample is to be run in a cup: Place the filled, labeled sample cup on a sample cup rack. With the cursor in the desired tube position press the parameter keys to select the tests to be run. Press the ID No. Entry key and enter the sample ID on the keypad. Then press Enter on the keypad.

    d. Press Start

    e. Indicate if you wish the instrument to start at the first tube (this is the only option after the machine has been turned off) or continue through the reaction rube rack from the previous spot.

f. The analyzer will read the barcode or assign the manually entered sample ID to the specimen, aliquot the sample, and perform ordered tests.

    1. When the instrument is done sampling the specimen, you will be allowed to open the sample drawer and add specimens when the instrument displays "Replace Rack? YES"

RESULTS

Reference Ranges:

Therapeutic

0.3-0.7 IU/ml (Unfractionated Heparin)

0.6 - 1.0 IU/mL (Low Molecular Weight Heparin)

Prophylaxis

0.2 - 0.5 IU/mL (Low Molecular Weight Heparin)

Reportable Range: 0.10-1.50 IU/ml

LIMITATIONS

For information regarding interference by endogenous and exogenous substances please refer to chapter "Interferences".

Turbidity and particles in the samples may interfere with the assay. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10inutes at approximately 15000 x g) must be excluded from the assay.

Due to matrix effects, inter-laboratory survey samples and control samples may yield results that differ from those obtained with other methods. It may therefore be necessary to assess these results in relation to method-specific target values.

Siemens Healthineers has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Users defined modification are not supported by Siemens Healthineers as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Healthineers Application Sheets or the Instructions for Use.

REFERENCES

Package insert INNOVANCE Heparin Assay