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|  | PROCEDURE | CS-Marina del Rey Hospital Clinical Laboratory & Pathology Marina del Rey Hospital 4690 Lincoln Blvd. 90292 |
| | COAGULATION | Effective Date: 11.20.12 |
| | | Revision Date: 03.21.19 |
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| Approved by: <u>James Keefe, MD</u> Medical Director Clinical Laboratory | Date Signed: <u>4/3/2019</u> | |
| Title: | INNOVANCE D-DIMER ON CA1500 | |

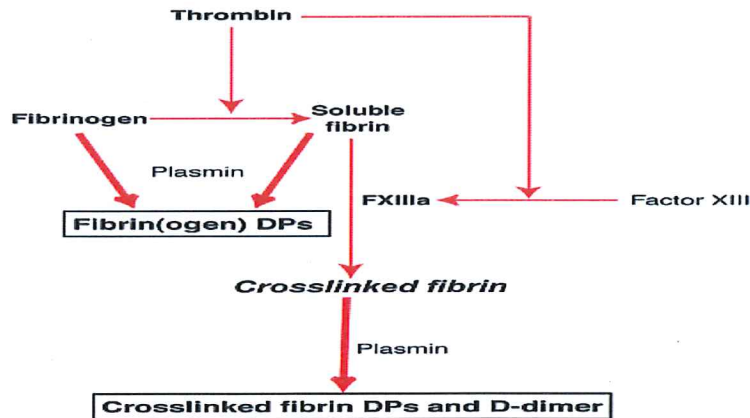
I. INTENDED USE

4/3/19

The Innovance® D-dimer assay is intended for use in conjunction with a nonhigh clinical pretest probability (PTP) assessment model to exclude deep vein thrombosis (DVT) and pulmonary embolism (PE).⁶ This test can be used to exclude VTE with nonhigh pretest probability (ie, low or low/moderate pretest probability). In an exclusion strategy, a D-dimer below the established threshold in a nonhigh pretest probability patient does not require further testing to exclude VTE.

II. CLINICAL SIGNIFICANCE

The Advanced D-Dimer is intended for use as an aid in the diagnosis of venous thromboembolism (VTE), deep vein thrombosis (DVT), or pulmonary embolism (PE).



Coagulation activation results in the cleavage of fibrinogen to fibrin. The resulting fibrin molecules spontaneously aggregate and are cross-linked by Factor XIII producing a fibrin clot. In response to the coagulation process the fibrinolytic system is activated resulting in the conversion of plasminogen into plasmin, which cleaves fibrin (and fibrinogen) into the fragments D and E. Due to cross-linkage between D-domains in the fibrin clot, the action of plasmin releases fibrin degradation products with cross-linked D-domains. The smallest unit is D-dimer. Detection of D-dimers, which specifies cross-linked fibrin degradation products generated by reactive fibrinolysis, is an indicator of coagulation activity. Fibrin degradation products are not consistently "D-dimer" but are a mixture of fragments and complexes of different molecular weight.

The presence of D-dimer confirms that both thrombin and plasmin have been generated since it can only be produced as the result of the plasmin degradation of cross-linked fibrin. The in vivo half-life of D-dimer is approximately eight hours.

| | | |
|--------|--------------------------------------|---------|
| | POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 | |

Elevated D-dimer levels are observed in all diseases and conditions with increased coagulation activation, like thromboembolic disease, DIC, acute aortic dissection, myocardial infarction, malignant diseases, obstetrical complications, third trimester of pregnancy, surgery, or polytrauma. However, in the context of venous thromboembolism, symptoms being present since a certain period, eg, longer than a week, may produce normal D-dimer values.

For the diagnosis of DIC a scoring system has been suggested, in which elevated D-dimer levels represent the major indicator of DIC. While increased levels of D-dimer are not specific for DVT or PE, low D-dimer levels may be used to rule out these conditions. The negative predictive values for DVT and PE are approaching 100% for the Innovance D-dimer assay employing a cutoff of <0.5 mg/L FEU. The negative predictive value is further enhanced using a clinical probability model along with D-dimer in the decision process. Values less than 0.5 mg/L FEU in an individual with a low clinical risk of venous thrombosis can serve as the basis for not performing more expensive diagnostic tests for DVT and PE. Patients with results greater than this cutoff require further diagnostic testing to establish the diagnosis.

III. PRINCIPLE

Polystyrene particles covalently coated with a monoclonal antibody (8D3)10 are aggregated when mixed with samples containing D-Dimer. The D-Dimer cross-linkage region has a stereo symmetrical structure, i.e., the epitope for the monoclonal antibody occurs twice. Consequently, one antibody suffices to trigger an agglutination reaction, which is then detected turbidimetrically via the increase in turbidity. On the CA-1500, quantitative results are related to a calibration curve, which is prepared automatically by dilution of standard plasma.

IV. SPECIMEN

Mix nine parts of freshly collected blood with one part of 0.11 mol/L (3.2%) sodium citrate anticoagulant, avoiding the formation of foam.

Invert the tube gently three or four times immediately after venipuncture to ensure proper mixing of blood and anticoagulant.

- A syringe or evacuated tubes (blue top) may be used with caution for collection.
- If multiple specimens are collected, the coagulation sample should be the second or third tube collected. If blood is drawn from an indwelling catheter, the line should be flushed with 5.0 ml saline and the first 5.0 ml of blood or six dead space volumes of the catheter discarded or used for other laboratory tests.
- The citrate concentration must be adjusted in patients who have hematocrit values above 55%. Specimens that are clotted, collected in the wrong tube, have visible hemolysis or have less than the expected fill should be rejected.

V. SPECIMEN HANDLING

- Checked the blue top for clot formation by gentle inversion.
- Centrifuge the blood specimen for a minimum of 10 minutes at 1500 ref (x g) within 2 hours after collection.
- Patient plasma should be tested within 4 hours if stored at room temperature, up to one month at

| | | |
|--------|--------------------------------------|---------|
| | POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 | |

18°C.

- If immediate testing is to be done, the plasma may remain on the packed cells or separated. To separate plasma, use a plastic transfer pipette, remove the plasma to a plastic tube. If testing is not complete within 4 hours or if shipment is required, the plasma may be stored frozen at 18°C for up to one month. Frozen plasma samples must be thawed within 10 minutes at 37°C and tested within 2 hours after thawing. Thaw plasma only once.

VI. INSTRUMENTATION

Siemens Sysmex® CA-1500 Coagulation Analyzers.

VII. EQUIPMENT

- Reaction tubes.
- Sample plates.
- Waste and rinse bottles.
- Pipettes to deliver 4 ml and 1 ml.
- Reagents
 - Innovance D-Dimer Kit containing:
 - Siemens Innovance D-Dimer Reagent
 - Siemens Innovance D-Dimer Buffer
 - Siemens Innovance D-Dimer Supplement
 - Siemens Innovance D-Dimer Diluent
 - Siemens Innovance D-Dimer Calibrator
 - Empty bottles for aliquoting reagent, diluent, supplement and buffer.
 - Siemens CA System Buffer or Owrens Veronal Buffer (B4265-34)
 - CA Clean I (B4265-1)
 - Preservative-free distilled or deionized water

▪ Reagent Preparation, Storage and Stability

All components of a kit are lot-specific. Do not combine lots.

Follow the preparation instructions prior to use according to Table 1.

Table 1. Instructions for the preparation of the kit components

| Instructions | Innovance D- Dimer Reagent | Innovance D-Dimer Buffer/Supplement/ Diluent | Innovance D-Dimer Calibrator |
|--------------------------------|---|--|---|
| Reconstitution | <ol style="list-style-type: none"> 1. Dissolve with 4.0 ml distilled water 2. Invert 3 times 3. Leave the vial for at least 15 minutes at 15 -25 ° C | READY FOR USE | <ol style="list-style-type: none"> 1. Dissolve with 1.0 ml distilled water. 2. Mix carefully without foam formation. 3. Leave the vial for at least 15 minutes at 15 -25 ° C |
| Prior to placing on the system | <ol style="list-style-type: none"> 1. Mix well (again) by inverting 3 times 2. Avoid foam formation 3. Remove bubbles | <ol style="list-style-type: none"> 1. Mix carefully 2. Avoid foam formation 3. Buffer only: resuspend potential precipitates by gently swirling. Any residual precipitates after resuspension do not impact test results 4. Remove bubbles | <ol style="list-style-type: none"> 1. Mix (again) carefully 2. Do not use if vial contains visible clot |

POLICY & PROCEDURE MANUAL

CS-MDRH

TITLE:

INNOVANCE D-DIMER ON CA1500

| Instructions | Innovance D- Dimer Reagent | Innovance D-Dimer Buffer/Supplement/ Diluent | Innovance D-Dimer Calibrator |
|-----------------------|--|---|---|
| Aliquoting | Mix well (again) by inverting 3 times | 1. Aliquot into an empty vial provided with the same kit. | N/A |
| | 2. Aliquot into an empty vial provided with the same kit | 2. Discard empty vials if unused until complete consumption of the kit. | |
| | 3. Discard empty vials if unused until complete consumption of the kit. | | |
| Freeze and thaw | <ol style="list-style-type: none"> 1. Use the original container or the empty vial provided with the same kit 2. Follow storage instructions in the section "Storage and Stability: 3. Thaw at 37 cc within 10 minutes. Thereafter the vial may no longer be stored at 2 - 8 cc. 4. Do not freeze again after thawing. | | Refer to section "Storage and Stability" |
| Placing on the system | Place Innovance D- Dimer Reagent in Reagent Holder position B1. | <ol style="list-style-type: none"> 1. Place Innovance D-Dimer Buffer in Reagent Holder position B2. 2. Place Innovance D-Dimer Supplement in Reagent Holder position B4. 3. Place Innovance D-Dimer Diluent in Reagent Holder position D6. | Place Innovance D-Dimer Calibrator in Sample Rack position I. |
| <i>Note</i> | <i>The reconstitution, opening, or freezing date may be noted on the vial label using the framed free space.</i> | | |

Storage and Stability:

The kit may be used up to the expiry date indicated on the label if stored unopened at 2 - 8 °C.

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|--------------------------------------|------------------------------------|
| POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 |

Table 2: Stability after reconstitution or first opening (closed vial)

| Temperature | Innovance D-Dimer Reagent | Innovance D-Dimer Buffer | Innovance D-Dimer Supplement | Innovance D-Dimer Diluent | Innovance D-Dimer Calibrator |
|-------------------|---------------------------|--------------------------|------------------------------|---------------------------|------------------------------|
| 2 -8 ° C | 4 weeks | 4 weeks | 4 weeks | 4 weeks | - |
| ≤ -18 °C | 4 weeks | 4 weeks | 4 weeks | 4 weeks | - |
| 15 - 25 °C | - | - | - | - | 4 hours |
| Onboard stability | 24 hours | 24 hours | 24 hours | 24 hours | |

Notes:

Do not refreeze and thaw. Follow freeze and thaw instructions in section "Preparation of the Reagents".

Information about on-board stability is specified in the Reference Guides (Application Sheets).

Indication of Deterioration: No evidence of vacuum in vial upon opening, difficulty in reconstituting reagents and controls outside acceptable limits.

Materials Needed but not Provided in Reagent Kit

Siemens Innovance D-Dimer Controls (levels 1 and 2)

Both controls are lyophilized preparation of pooled plasma, supplemented with a standardized reference D-Dimer preparation, stabilized with 5-chloro-2- methyl-4-isothiazole-3-one, 2-methyl-4-isothiazole-3-one (<1 mg/L) and Sodium Azide.

Control Preparation

1. Reconstitute the Innovance D-Dimer Control Plasma 1 and 2 with 1.0 ml of deionized water.
2. Re-stopper vial and shake carefully to dissolve (without foam formation).
3. Let stand at +15 to +25°C for at least 15 minutes before use Mix carefully once before using.

Control Stability after reconstitution (closed vial)

1. Stable for 7 days stored at 2-8°C.
2. Stable for 8 hours at 15-25°C
3. Stable for 4 weeks frozen at ≤18°C. Controls must be stored in original containers. Do not refreeze after thawing.

CA Clean I

Preparation: A Clean I- liquid and ready for use

Storage and Stability

- Store at 2-8°C. Do Not Freeze.
- Stable unopened at 2-8°C until expiration date on bottle.
- Opened bottle stable for 30 days at 2-8°C.

CA System (Owrens Verona! Buffer)

Preparation: Owrens Verona! Buffer is liquid and ready for use

Storage and Stability

- Store at 2-8°C. Do Not Freeze.
- Stable unopened at 2-8°C until expiration date on bottle.
- Opened bottle stable for 30 days at 2-8°C.

| | | |
|--------|--------------------------------------|---------|
| | POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 | |

VIII. PROCEDURE

Refer to the Testing Procedure section of the CA-1500 Operators Manual for step-by-step procedure of Loading Reagents (when instrument is not in operation mode), Loading Consumables, Discarding Waste material, Replenish Reaction tubes, Replenish Rinse Solution, Dispose of Liquid Waste (note: Confirm that the instrument is not operating or has been interrupted due to waste is full), and Dispose of Used Reaction Tubes from the Trash Box.

Calibration/Calibration Verification:

- Calibration verification must be performed every 6 months with the same lot when indicated by Qc data, after maintenance and or when, recommended by the manufacturer
- Calibration provides instrument specific curves and compensates for minor variation in the assay throughout the life of the kit.
- Verification of calibration is performed by analyzing the controls and the calibrator with the new calibration curve.
- The controls should be within the ranges and the calibrator within 10% of the stated value.
- Recalibration is done if values obtained are not in range. Additionally, three patient's samples are run on the current curve and the new curve for correlation.

Calibrating from a Sample Rack:

1. From the Main Menu, press [Standard Curve].
2. Press [Select Test].
3. Press [DDi] to display 'Standard Curve' screen.
4. Press [Lot No. Entry].
5. Select Reagent1 and Reagent 2.
6. Enter lot number. Press [Enter], [Quit].
7. Select [Standard Analysis].
8. Enter the assay value of the calibrator from the Innovance D-dimer calibrator lot number package insert.
9. Press [Enter].
10. Verify that the dilution set is correct. Press [Select Oil. Set] until dilution set #1 appears at the upper left corner.
11. Select 2 replicates for each dilution.
12. Press [Start] key to start analysis for the Standard. The message
13. "Analyzing" will be displayed. When the samples have been aspirated, the "Replace Rack? Yes" message will appear.
14. Check that the Standard Curve is acceptable. If acceptable, press [Fix] to store the new standard curve.

Printing the Standard Curve

- From the Main Menu press [Standard Curve], Select the "DDi" key for Innovance D-dimer assay.
 - From the Standard Curve screen, press [More];
 - press [Output Input] to display the Standard Curve Output Input menu;
 - press [Output]; PRESS [GP Print] to display the Print Confirmation message window; press [Output];
 - press [OK] to start printing; press [OK] to return to the Main Menu or press [Return] when printing is complete to remain at the Standard Curve screen.
- necessary.

| | | |
|--------|--------------------------------------|---------|
| | POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 | |

Saving the Standard Curve to a Diskette if needed

At this facility, the curve is stored on the instrument only. The analytical measurement range is validated by running low control, high control and the D-dimer calibrator. Low and high control values must be within manufactures control range, D-dimer calibrator must correlate within 5% of manufactures value. Additionally, three patient samples ran on the prior calibration curve are repeated on the new curve and should correlate within 10%. The new calibration curve is not accepted if this validation does not pass criteria. Repeat calibration, troubleshoot or call Siemens technical support if

Sample Processing

Refer to the CA-1500 Coagulation System Procedure, "Sample Processing" section for details for details in loading and ordering samples and controls.

Control Processing

1. From the Main Menu, Press [ID NO. ENTRY]
2. Press the [QC] key on the numeric keypad and the quality control file; e.g., for QC file five, press [QC] AND [5].
3. Press the [DDi] key.
4. Press [Enter]. The Worklist will display the ID as quality control file number; e.g., "QC05".
5. Place the control material (Innovance D-Dimer Controls levels 1 and 2) in a sample rack.
6. If necessary, continue to program routine patients or other samples. When entry is complete, press [Start].

Reporting Results

The D-dimer concentration in mg/L is calculated automatically by the analyzer based on the reference curve. The D-dimer level is expressed as initial fibrinogen equivalent unit (FEU). An FEU is the quantity of fibrinogen initially present that leads to the observed D-dimer level. Increases in D-dimer concentration observed with thromboembolic events can be variable due to localization, extension and age of the thrombus. Therefore, a thromboembolic event cannot be excluded with certainty solely based on a D-dimer concentration being within the reference range of ostensibly healthy persons.

Verify results in Meditech.

Quality Control values are transmitted and accepted in UNITY.

Record results on the worksheet, patient requisition (if applicable) and in the computer system.

Record quality control values. Hemolyzed, lipemic, or icteric samples must be noted with the result.

Reference Interval

At this facility the Reference interval values determined for D-Dimer are 0.50-2.00mg/L.

At this facility, results are accompanied by a "Reference Range Comment":

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| <0.5 | Negative |
| 0.5 to 2.0 | Possible Thrombotic Event |
| >2.0 | Suggestive of DIC |

Procedural Notes

Overall performance of D-Dimer testing is dependent on reagent and instrument performance. Acceptable total coefficient of variation (CV) of the analytical system is less than 20% (equivalent to 5% SD) on the same lot of

| | |
|--------------------------------------|------------------------------------|
| POLICY & PROCEDURE MANUAL | CS-MDRH |
| TITLE: | INNOVANCE D-DIMER ON CA1500 |

control plasma.

A new reference curve should be established every six months, when using a new lot of reagents, new instrument or when results of controls (or proficiency samples) are out of range.

Limitations

The analytical range is approximately 0.19 to 4.40 mg/L FEU. The measuring range can be extended to approximately 35.20 mg/L FEU by automatic re-dilution of samples above 4.40 mg/L FEU.

All components of the Innovance D-Dimer kit are lot dependent. The combination of lots may lead to incorrect results.

Interferences

Levels of the following do not appear to interfere with the Innovance D-Dimer assay:

| Substance | Levels Up to |
|-------------------|--------------|
| Bilirubin | 12mg/dL |
| Hemoglobin | 200 mg/dL |
| Triglycerides* | 400 mg/dL |
| Heparin | 3.0 IU/ml |
| Rheumatoid Factor | 1330 IU/ml |
| Creatinine | 30 mg/dL |
| Albumin | 6 g/L |
| Fibrinogen** | 1000 mg/dL |
| Urea | 500 mg/dL |
| Uric Acid | 20 mg/dL |
| Immunoglobulin G | 5 g/dL |

*Higher levels of lipids or turbid samples can lead to falsely elevated or decreased values.

**Higher levels of fibrinogen or fibrinogen degradation product fragment D can lead to falsely elevated or decreased values.

Backup Procedure

At this facility, there are two CA-1500 systems to backup for each other to minimize downtime. When either instrument is not operational, call Siemens technical support at 800-242-3233 for technical assistance over the phone. In the mean time, use the alternate CA-1500 for patient testing. The technical representative will service the instrument Monday through Friday from 8 a.m. to 5 p.m. If both instruments are not operational, technical service will also be provided after hours.

IX. REFERENCES

1. Siemens Innovance D-Dimer Reagent package insert, Siemens, Marburg, Germany, 2009.
2. Siemens Innovance D-Dimer Application Sheet, Siemens, Marburg, Germany, June 2011.
3. Siemens CA-1500 Operator's Manual, 2003.
4. Siemens Innovance D-Dimer Instruction for Use reference Guide.CA-1500 validation data.
5. Clinical Laboratory Standards Institute. Collection, transport and processing of blood specimens for coagulation testing and general performance of coagulation assays. Approved Guideline, 3rd Edition. NCCLS Publication H21-A3. Wayne, PA, 1998.

