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DOCUMENT NUMBER:	RIV-PPP-1093
DOCUMENT TITLE:	Assay of D-Dimer on the STA_ Analyzers
DOCUMENT NOTES:	

LOCATION:	RIV-rel	**VERSION:**	02
DOC TYPE:	RIV PPP	**STATUS:**	Release

EFFECTIVE DATE:	18 Apr 2024	**NEXT REVIEW DATE:**	18 Apr 2026
RELEASE DATE:	18 Apr 2024	**EXPIRATION DATE:**	

AUTHOR:		**PREVIOUS NUMBER:**	
OWNER:		**CHANGE NUMBER:**	RIV-CR-0383

Assay of D-Dimer on the STA® Analyzers

Purpose

This procedure provides instructions for D-dimer testing on STA-R Max® and/or STA Compact Max®.

Scope

This procedure is intended for trained Clinical Laboratory Scientists and Medical Laboratory Technicians (MLT) working in the coagulation section.

Principle

This assay is based on the change in turbidity of a microparticle suspension that is measured by photometry. A suspension of latex microparticles, coated by covalent bonding with monoclonal antibodies specific for D-dimer, is mixed with the test plasma whose D-dimer level is to be assayed. An antigen-antibody reaction takes place, leading to an agglutination of the latex microparticles which induces an increase in turbidity of the reaction medium. This increase in turbidity is reflected by an increase in absorbance, the latter being measured photometrically. The increase in absorbance is a function of the D-dimer level present in the test sample.

The **STA® - Liatest® D-Di** kit is an immuno-turbidimetric assay for the quantitative determination of D-dimer in venous plasma (in 3.2 % sodium citrate) for use on STA® line of analyzers. The STA® - Liatest® D-Di is intended for use in conjunction with a clinical pretest probability (PTP) assessment model to exclude pulmonary embolism (PE) and deep venous thrombosis (DVT) in outpatients suspected of PE or DVT.

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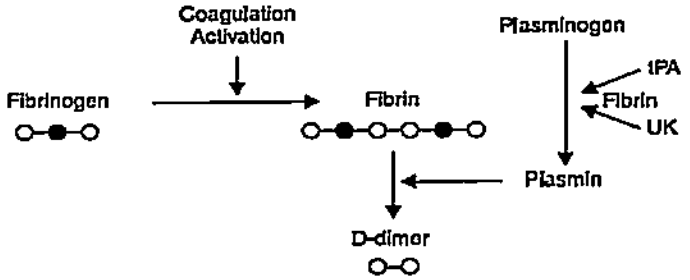
Assay of D-Dimer on the STA® Analyzers, Continued

Principle, continued

The specific degradation of fibrin (i.e., fibrinolysis) is the reactive mechanism responding to the formation of fibrin.
Plasmin is the fibrinolytic enzyme derived from the inactive plasminogen. Plasminogen is converted into plasmin by plasminogen activators. The main plasminogen activators are the tissue plasminogen activator (tPA) and the pro-urokinase which is activated into urokinase (UK) by, among others, the contact system of coagulation.
In the bloodstream, plasmin is rapidly and specifically neutralized by alpha2-antiplasmin thereby restricting its fibrinogenolytic activity and localizing the fibrinolysis on the fibrin clot.
On the fibrin clot plasmin degrades fibrin into various products. Antibodies specific of these products, which do not recognize fibrinogen, have been developed. The presence of these various fibrin degradation products, among which D-dimer is the terminal product, is proof that the fibrinolytic system is in action in response to coagulation activation.



The Degradation of Fibrin into D-dimer

Specimen sources

Citrated blood collected in 9:1 ratio with anticoagulant of 3.2% sodium citrate.

Specimen collection, processing, transport, and storage

- Sample collection must be in conformity with the recommendations for hemostasis tests. See procedure for "Specimen Requirements for Coagulation Studies".
- Centrifuge the specimen tube at a speed and time required to produce platelet-poor plasma.
- If testing cannot be performed within 8 hours, the plasma must be frozen at a temperature of less than minus 20°C until testing can be performed.
- Frozen specimen is stable for four weeks at –20 °C. Thaw the sample at 37°C; allow sufficient time to obtain complete thawing.

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Assay of D-Dimer on the STA® Analyzers, Continued

Specimen rejection

Reject the following samples under unacceptable conditions:

- Improperly labeled
- Short draws and overdraws
- Clotted
- Drawn in wrong tube
- Visible hemolysis
- Unspun greater than four (4) hours since time of collection

Equipment

- STA-R Max® and/or STA Compact Max®
- Centrifuge

Materials and Supplies

- Pipettes
- Pipette tips
- STA® Mini reducer
- Cuvette Roll-1000
- Reagent Grade Water

Safety

Exercise great care in the handling of reagent and patient samples.
Waste disposal must be performed in accordance with the applicable local regulations.

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Reagents and/or Media

The following contains the list of reagents and/or media required:

1. **STA® - Liatest D-Di kit** (REF 00515): contains two types of reagent vials. Take care to use only the reagents from the same kit or the same lot.
   - **Reagent 1:** Tris buffer
   - **Reagent 2:** Latex suspension of microlatex particles coated with two different mouse monoclonal anti-human D-dimer antibodies (8D2 and 2.1.16) then stabilized with bovine albumin.

   1.1 Storage: Proper storage at 2-8°C allows the reagents in intact vials to be stable until the expiration date indicated on the box label.

   1.2 Preparation:
   a. From storage at 2-8°C, allow the reagent to stand at room temperature (18-25°C) for fifteen (15) minutes before reconstitution.
   b. Mix the reagents by gentle swirling of the vials without creating any bubbles.
   c. Remove the rubber stopper on the vial, then insert a new STA® Mini reducer, and place the perforated cap on each vial.
   d. Follow local protocol in documenting reagent expiration date.

   1.3 Stability: With the STA® Mini reducer and perforated cap in place, the stability of both Reagents 1 and 2 after opening and in their original vials is 15 days on the STA-R Max® and Compact Max®.

   1.4 Loading: When the reagent is ready for use, load into the instrument according to the recommendations of the reference manual of the analyzer model. The vial position in the instrument is the following:
   - On the STA-R Max® model, place in a position in the R1 area of the product drawer.
   - On the STA Compact Max® model, place in a position of the product drawer.

2. **STA® - Owren-Koller** (REF 00360) is a ready-to-use buffer solution intended for use as a diluent for reagents and patient samples in coagulation tests. It has a pH of approximately 7.35.

   2.1 Storage: Proper storage at 2-8°C allows the reagent in intact vial to be stable until the expiration date indicated on the box label.

   2.2 Preparation:
   a. From storage at 2-8°C, allow the reagent to stand at room temperature (18-25°C) for thirty (30) minutes beforeuse.
   b. Open the buffer bottle. Do not install either an STA® reducer or a perforated cap on the buffer bottle.

   2.3 Stability: After opening, it remains stable for:
   - Three (3) days on the STA-R Max® and Compact Max®

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Assay of D-Dimer on the STA® Analyzers, Continued

- In case of partial use, the remaining solution stored at 2-8°C in its original bottle with the cap on top, is stable, when free of any contamination, until the expiration date indicated on the bottle.

2.4 Loading: After opening the buffer bottle, load into the instrument according to the recommendations of the reference manual of the analyzer model. The vial position in the instrument is the following:

- On the STA-R Max® model, place in a position in the R0 area of the product drawer.
- On the STA Compact Max® model, place the bottle in the sample drawer.

The buffer solution is automatically used by the instrument.

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Assay of D-Dimer on the STA® Analyzers, Continued

Quality Control

STA – Liatest Control N + P kit (REF 00526): kit containing assayed normal and abnormal plasmas intended for the quality control of the antigenic assays by the immune-turbidimetric method on STA-R Max® and STA Compact Max® analyzers including D-dimer.

- **Reagent 1:** STA® - Liatest Control, citrated normal human plasma, lyophilized.
- **Reagent 2:** STA® - Liatest Control, citrated abnormal human plasma, lyophilized.

An Assay Value insert with two barcodes, one for each control level, is provided in the box. Each barcode contains the following information: lot number, kit code number, reagent code number, expiration date and parameter values determined with analyzers of the STA® line for the relevant lot.

A. Storage: Proper storage at 2-8°C allows the controls in intact vial to be stable until the expiration date indicated on the box label.
B. Preparation:
   1. Allow the control vials to stand at room temperature (18-25°C) for thirty (30) minutes before reconstitution.
   2. Reconstitute each vial of Reagent 1 or 2 with exactly 1 mL of reagent grade water.
   3. Allow the reconstituted material to stand for 30 minutes at room temperature.
   4. Swirl vials gently before use.
C. Stability: Once reconstituted, Reagents 1 and 2 remain stable for 8 hours on the STA-R Max® and Compact Max®.
D. Loading: When Reagents 1 and 2 are ready for use, load into the instrument according to the recommendations of the reference manual of the analyzer model. The vial position in the instrument is the following:
   - On the STA-R Max®, place the control vials in the R0 area of the product drawer.
   - On the STA Compact Max® model, place the control vials in one of the positions 1 to 18 or 35 to 38 of the product drawer.

Follow instrument model's start-up and basic procedure to run controls.

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Assay of D-Dimer on the STA® Analyzers, Continued

Quality Control Policy

- Two different levels of controls must be run every eight hours of patient testing and upon reagent changes to ensure accuracy and reproducibility of the results.
- Control results must be within acceptable limits prior to validating results.
- Ensure that the values obtained for the control assays are within the ranges stated in the control box.
- When quality control acceptable limits are exceeded, check all components of the test system and corrective action must be taken according to local protocol before validating patient results.

Calibration/ Verification

- The D-Dimer reagent kits are pre-calibrated: this calibration is identical for all the reagent of each lot.
- When the operator scans a new lot of Liatest D-Dimer reagent, the STA® analyzer will request the operator to scan the bar code printed on the bar code insert across the analyzer bar code reader.
- The calibration values for the lot of reagents being used will subsequently be validated after the two D-dimer control levels have been determined.
- The calibration curve can be examined on the screen of the analyzer in the "Calibration" menu.

Procedure

Follow the steps below to have the specimen tested for D-dimer assay for the D-dimer test order received:

Step	Action
1	Load the patient plasma undiluted on to the analyzer. Refer to the analyzer basic start-up and operation procedure on loading and testing samples.
2	The analyzer will automatically run the D-dimer assay if barcode is readable by the analyzer upon loading, and the host is up. Note: • If barcode wasn't read by the analyzer, and the host is up, manually enter the specimen accession number. The test will be downloaded from the host. • If barcode was read and the communication to the host is down, select the D-dimer assay to be run.
3	If any of the patient results falls outside the working range of the assay, the instrument automatically retests the sample in question at an appropriate dilution provided that the procedure with dilution has been chosen.

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Assay of D-Dimer on the STA® Analyzers, Continued

Method Specifications

The limit of detection on STA-R Max® and STA Compact Max® is 0.27 mcg/ml FEU).

With autodilution of the sample, the assay working range is 0.27 - 20.00 mcg/ml (FEU).

Unit of Measure

The analyzer automatically plots the results in delta OD off a standard curve and is reported as mcg/mL FEU (FEU- Fibrinogen Equivalent Units) as unit of measure.

Reference Range

The table below represents the reference range for each D-Dimer test order:

Order Code	Assay Description	Age	Reference Range
D-Dimer DIC	D-Dimer DIC Quant	0 to 250 years	< 0.50 mcg FEU/mL (displays as <= 0.49 mcg FEU/ml in Cerner)
D-Dimer DVT	D-Dimer DVT Quant	0 to 250 years	< 0.50 mcg FEU/mL (displays as <= 0.49 mcg FEU/ml in Cerner)
D-Dimer PE	D-Dimer PE Quant	≤50 years	< 0.50 mcg FEU/ml (displays as <= 0.49 mcg FEU/ml in Cerner)
		51 – 79 years	< (Age divided by 100) mcg FEU/ml
		≥ 80 years	< 0.80 mcg FEU/ml (displays as <= 0.80 mcg FEU/mL in Cerner)

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Assay of D-Dimer on the STA® Analyzers, Continued

Reporting

The table below represents the reportable limit range:

Order Code	Assay Description	Reportable Limits	
		Low	High
D-Dimer DIC	D-Dimer DIC Quant	0.27	20.0
D-Dimer DVT	D-Dimer DVT Quant	0.27	20.0
D-Dimer PE	D-Dimer PE Quant	0.27	20.0

Report results less than 0.27 as <0.27 mcg/mL FEU.
Report Vmax after 1:5 auto dilution and greater than 20.00 results as >20.00 mcg/mL FEU.

Clinical Significance

Clinical applications for this test are as follows: Disseminated Intravascular Coagulation (DIC), negative predictor for the diagnosis of a thrombotic episode (i.e., DVT, PE), efficacy of treatment for a thrombotic episode and screen for possible re-occurrence (MI), and screen for other activation states of coagulation (i.e., post-operative, cancer, cirrhosis).

Limitations

1. The STA® - Liatest® D-Di is insensitive to the following substances: hemoglobin (up to 2 g/l), conjugated bilirubin (up to 290 mg/l), unconjugated bilirubin (up to 200 mg/l), unfractionated heparin (up to 2 IU/ml), and low molecular weight heparin (up to 2 anti-Xa IU/ml).
2. Cloudy plasmas may lead to an under-estimation of the D-Dimer level. Test order can be rejected when instrument generates error from interference.
3. An over-estimation of D-Dimer level may be seen in the following conditions; FDP concentrations greater than 15 *u*g/ml, the presence of rheumatoid factor at a level greater that 50 IU/ml, and the presence of anti-bovine albumin and/or anti-mouse antibodies in certain subjects.

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Assay of D-Dimer on the STA® Analyzers, Continued

Non-Controlled Documents

The following non-controlled documents support this procedure.

STA®-LIATEST DDI Immuno-Turbidimetric Assay of D-Dimer (REF 00515) by STA® Analyzers. Diagnostica Stago Package insert, May 2021.

STA® - Liatest Control N + P kit (REF 00526): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA® Analyzers package insert. Ver. English 2- July 2017.

STA® - Owren-Koller Buffer (REF 00360) Buffer Solution for Coagulation Testing. Package insert. English –March 2022.

Controlled Documents

The following controlled documents support this procedure.

Stago STA-R Max® Start Up and Basic Operations Procedure
Stago Compact Max® Start Up and Basic Operations Procedure
Specimen Requirements for Coagulation Studies
Approved SCAL Coagulation Autoverification Rules

Author(s)

SCPMG Coagulation Working Group

Signature Manifest

Document Number: RIV-PPP-1093 **Revision:** 02
Title: Assay of D-Dimer on the STA_ Analyzers
Effective Date: 18 Apr 2024

All dates and times are in Pacific Standard Time.

Assay of D-Dimer on the STA_ Analyzers

Operations Director Approval

Name/Signature	Title	Date	Meaning/Reason
Annaleah Raymond (Q741709)	Laboratory Operations Director	14 Apr 2024, 06:58:29 PM	Approved

Medical Director Approval

Name/Signature	Title	Date	Meaning/Reason
Mark Taira (P161328)	CLIA Director	16 Apr 2024, 05:27:51 PM	Approved