mini Vidas D-Dimer Exclusion II Assay

PRINCIPLE / PURPOSE: Fibrin degradation products (FbDP) are a highly heterogeneous group of soluble fragments that appear in the circulation as a result of two simultaneous physiological processes:

* Coagulation, resulting in the conversion of soluble fibrinogen into insoluble stabilized fibrin by the enzymes thrombin and factor XIIIa,
* Fibrinolysis, resulting in the dissolution of the fibrin clot by the enzyme plasmin. The D-Dimer fragment is the terminal product of this process.

Although FbDP’s vary in size, they are characterized by the presence of one or more D-Dimer epitopes. Therefore, FbDP’s are collectively referred to as ‘D-Dimer’.

The mini-Vidas D-Dimer Exclusion II Assay is an automated quantitive test for use on the mini Vidas instrument. The immunoenzymatic determination of fibrin degradation product (FbDP) in human plasma (sodium citrate, CTDA) using ELFA technique (Enzyme Linked Fluorescent Assay). Mini Vidas D-Dimer Exclusion II is indicated for use in conjunction with a clinical pretest probability assessment model to exclude deep vein thrombosis (DVT) and pulmonary embolism (PE) disease in outpatients suspected of DVT or PE.

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase with an anti-FbDP monoclonal antibody adsorbed on its surface as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed single-use reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR® several times. First the sample is taken by the SPR®, diluted and then cycled in and out of the SPR® several times. The antigen binds to the anti-FbDP immunoglobulins coated on the SPR®. Unbound components are eliminated during a washing step. During the second step, the conjugate, which contains an alkaline phosphatase-labeled anti-FbDP monoclonal antibody, binds to the antigen coated on the SPR® to form a “sandwich”. Unbound components are eliminated during the washing steps. A detection step is then performed. The substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®.

The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of the antigen present in the sample. Results are automatically calculated by the instrument in relation to the calibration curve stored in memory and a report is then printed.

SCOPE: This procedure provides instruction on D-Dimer testing performed on the mini Vidas instrument.

SPECIMEN:

Type: Collect blood by clean venipuncture in trisodium citrate (0.109 mol/L / 3.2%

or 0.129 mol/L / 3.8%) or CTAD (sodium citrate, theophylline, adenosine and

dipyridamole), observing the correct anticoagulant to blood ratio (1:9). Syringe collection is not recommended in order to avoid formation of micro clots in the sample.

Plasma samples separated from the pellet can be stored at 2 – 8 °C in stopper tubes for 3 days. The assay can be performed on plasma frozen for 6 months. Plasma must be frozen at -25± 6 °C immediately after decantation. It must be thawed rapidly at 37 °C when assaying and the assay should be performed rapidly after thawing. Two freeze/thaw cycles are acceptable.

INTERFERENCES

None of the following factors have been found to significantly influence this

assay:

 Hemolysis - after spiking samples with hemoglobin, up to 2 g/dL

 Lipemia - after spiking samples with lipids, up to 30 g/L equivalent in

triglycerides.

 Bilirubinemia - after spiking samples with bilirubin, up to 31.4 mg/dL

 Rheumatoid factor - up to 400 IU/ml (international units per

milliliter).

 Human albumin – up to 60 g/L.

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

EQUIPMENT AND MATERIALS:

Equipment: Biomerieux Mini Vidas Instrument

Materials:

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A. Reagents and Materials Provided

Refrigerate 2 to 8 oC Note: Do not freeze the reagent kit.

1. DEX2 Reagent Strips

2. DEX2 SPRs

3. DEX2 Calibrator (S1)

4. DEX2 Kit Controls (C1 and C2)

B. Reagent Preparation and Storage

1. DEX2 Calibrator - Reconstitute with 2 ml of distilled water. Wait 5

minutes and then mix (vortex). After reconstitution, the calibrator is stable

for 28 days at 2 to 8 oC or until the expiration date of the kit at – 25 + 6 oC

(Freeze immediately after reconstitution) 5 freeze/thaw cycles

are possible.

2. DEX2 Controls - Reconstitute with 2 ml of distilled water. Wait 5

minutes and then mix (vortex). After reconstitution, the controls are stable for

28 days at 2 to 8 0 C or until the expiration date of the kit at – 25 + 6 oC

(Freeze immediately after reconstitution) 5 freeze/thaw cycles are possible.

C. Materials Required But Not Provided

1. Calibrated pipette to dispense 200 ul and 2 ml.

2. Disposable pipette tips for pipette.

3. Powderless disposable gloves.

Preparation: Remove the required reagents and SPR's from the refrigerator. They can be used immediately. Use one DEX2 strip and one DEX2 SPR from the kit for each sample to be tested. Make sure the SPR pouch has been resealed after the required SPRs have been removed. Note: Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs.

Storage Requirements: Plasma samples separated from the pellet can be stored at 2-8 OC in stoppered tubes for 3 days. Samples that are not separated are stable at room temperature for 4 hours. The assay can be performed on frozen plasma for 6 months. Plasma must be frozen at -25+ 6 oC immediately after decantation. It must be thawed rapidly at 37 oC when assaying and the assay should be performed rapidly after thawing. Two freeze/thaw cycles are acceptable.

CALIBRATION:

Standard Preparation:

A. VIDAS® PTC Protocol Data Entry

When using the assay for the first time and before reading the Master Lot Entry (MLE) data, scan the barcode (at the end of the package insert) using the instrument’s

external barcode reader. This reading will allow the VIDAS® PTC

protocol data to be transferred to the instrument software for its update.

This data should only be read the first time the assay is used.

B. Master Lot Data Entry

Note: When using the assay for the first time, enter the VIDAS® PTC

protocol (barcode at the end of the package insert) before reading the

MLE data. If the MLE data have been read before the VIDAS® PTC

protocol, read the MLE data again.

Before each new lot of reagents is used, specifications (or factory master

calibration data) must be entered into the instrument using MLE data. If this operation is not performed before initiating the tests, the instrument will not be able to print results. The MLE should only be entered once for each lot.

It is possible to enter MLE data manually or automatically depending on

the instrument. For complete instructions refer to the Operator’s Manual.

C. Calibration

Calibration, using the calibrator provided in the kit, must be performed

each time a new lot of reagents are opened, after the master lot data has

been entered. Recalibration should then be performed every 28 days.

This operation provides instrument-specific calibration curve and

compensates for possible minor variations in assay signal throughout the

shelf life of the kit.

The calibrator, identified by S1, must be tested in duplicate in the same run.

The calibrator value must be within the set RFV "Relative Fluorescence Value"

range. If this is not the case, recalibrate.

QUALITY CONTROL:

Two controls are included in each VIDAS D-Dimer Exclusion II™ kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified as C1 and C2. Results cannot be validated if the control deviates from the expected value.

Note: Quality control must be performed daily as patient tests are run and resulted.

EXPECTED VALUES FOR THE CONTROLS AND

CALIBRATOR

The expected values for the controls and calibrator are printed once the MLE barcode is scanned. If the result from testing the controls and calibrator do not meet these specifications, do not report patient results.

PROCEDURE FOR RUNNING SAMPLES, CONTROLS, AND CALIBRATORS

1.Remove the required reagents from the refrigerator. They can be used

immediately.

2. Use one DEX2 strip and one DEX2 SPR from the kit for each

sample, control or calibrator to be tested. Make sure the SPR pouch has

been resealed after the required SPRs have been removed. Note:

Carefully reseal the pouch with the desiccant inside after use to maintain

stability of the SPRs.

3. Place the DEX2 strip and SPR on the miniVIDAS Preparation/Loading tray.

4. The test is identified by the “DEX2” code on the instrument. The

calibrator must be identified by “S1” and tested in duplicate. If the controls are to be tested, they should be identified by “C1” and “C2” and tested as a set.

5. If necessary, clarify samples by centrifugation.

6. Mix the calibrators, controls and samples thoroughly with a vortex-type

mixer in order to improve result reproducibility (for plasma separated

from the pellet).

7. Pipette 200 µl of calibrator, control, or sample into the well labeled VIDAS on the test strip.

8. Insert the “DEX2” SPRs and “DEX2” strips into the appropriate positions indicated on the instrument. Check to make sure the color labels with the assay code on the SPRs and the strips match.

9. Initiate the assay as directed in the Operator’s Manual. The instrument performs all the assay steps automatically.

10. Reclose the vials and return them to the freezer after pipetting.

11. The assay results will be completed within 20 minutes.

12. After the assay is completed, dispose of SPRs and strips into an appropriate receptacle.

INTERPRETATION & REPORTING RESULTS:

Reference Ranges:

Based on ARMC studies, the analytic measurement range of the mini Vidas D-Dimer Exclusion II is 61-7500 ng/ml (FEU).

A D-Dimer of > 500 ng/mL (FEU) is considered positive and a result of < 500 ng/mL (FEU) is considered negative.

Procedures for Abnormal Results:

Samples with D-Dimer concentrations <61ng/ml (FEU) and >7500 ng/ml (FEU) are reported as such. No dilutions or repeat testing is necessary in this case.

Reporting Format:

Once the assay is completed, the results are automatically calculated by the

instrument using the calibration curve that is stored by the instrument (4-parameter logistics model), and then printed. The concentrations are expressed in ng/ml FEU (Fibrinogen Equivalent Unit).

PROCEDURE NOTES:

Results are entered using the interface for the miniVidas.

1. Login to Sunquest
2. Select Result Entry
3. Enter the Instrument method code
4. Select the Result button at the bottom right
5. Use the binoculars at the bottom left to enter the container ID
6. Review results and Save
7. Accept results

Samples are stored in the rack with routine coagulation specimens.

LIMITATIONS OF THE PROCEDURE:

Interferences may be encountered with certain plasmas containing antibodies

directed against reagent components. For this reason, the VIDAS® D-Dimer

Exclusion II assay results should be interpreted taking into consideration the

patient’s history (clinical probability).

Clinical performance data were determined on an outpatient population.

Because D-dimer results are likely to be elevated in an inpatient population

due to stasis, chronic illness, post-surgery and other non-specific conditions

known to elevate D-dimer levels, the clinical utility of a negative result is not

likely to be realized in an inpatient population. Therefore, clinical performance results should not be extrapolated to an inpatient population.

SUPPLEMENTAL MATERIALS/ADDENDUM:

REFERENCES:

VIDAS System package insert. Refer to the insert for the complete details of the procedure, references, and performance of the product. January 2015

Thachil J, Fitzmaurice DA et al. Appropriate Use of D-Dimer in Hospital Patients. American Journal of Medicine; 2009; 9:17-19.

Mousa AY; Broce M. et al. Appropriate Use of D-Dimer Testing Can Minimize Over-Utilization of Venous Duplex Ultrasound in a Contemporary High-Volume Hospital. Annals of Vascular Surgery, 2014; 29(2): 1-7.

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| Medical Director Approval –  ARMC Cancer Center ARMC Main Lab |  |  |
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HISTORY PAGE

SOP Number: COAG-715

SOP Title: miniVidas D-Dimer Exclusion II Assay

Written By: Sharon Callahan

Manual in which Hard Copy of this SOP is located: ARMC Coagulation Manual

Distribution: ARMC Lab Coagulation Manual

Supersedes Procedure: N/A

SOP CHANGE CONTROL

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|  | Approvals | |  | Action | In |
| Mgmt. | Date | Director | Date |  | Effect |
| Q.Dyson | 5/4/17 |  |  | Updated worksheet for manual result entry | 5/4/17 |
|  |  |  |  |  |  |
| Q.Dyson | 8/29/17 |  |  | Update procedure format | 8/29/17 |
|  |  |  |  |  |  |
| Q.Dyson | 2/16/18 |  |  | Updated information on result entry to include interface | 2/27/18 |
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