1. **PRINCIPLE**

The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The sample is transferred into the wells containing anti-procalcitonin antibodies labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture is cycled in and out of the SPR® several times. This operation enables the antigen to bind with the immunoglobulins fixed to the interior wall of the SPR® and the conjugate to form a sandwich. Unbound compounds are eliminated during washing steps. Two detection steps are performed successively. During each step, 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) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to two calibration curves corresponding to the two detection steps. A fluorescence threshold value determines the calibration curve to be used for each sample. The results are then printed out.

1. **METHOD**

VIDAS® B·R·A·H·M·S PCT (PCT) is an automated test for use on the instruments of the VIDAS family for the determination of human procalcitonin in human serum or plasma (lithium heparinate) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. The VIDAS® B·R·A·H·M·S PCT (PCT) is intended for use in conjunction with other laboratory findings and clinical assessments to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock.

1. **REAGENTS AND MATERIALS**
	1. Reagents and Materials Provided

3.1.1 PCT Reagent Strips

3.1.2 PCT SPRs

3.1.3 PCT Calibrators (S1 & S2)

3.1.4 PCT Kit Controls (C1 and C2)

3.1.5 MLE Card

 Reagents and Materials should be refrigerated 2 to 8 C. Do not freeze the reagent kit.

* 1. Reagent Preparation and Storage
		1. PCT Calibrator - Reconstitute with 2 ml of distilled water. Wait 5 - 10 minutes and then mix. After reconstitution, the calibrator is stable for 8 hours at 2 to 8° C or until the expiration date of the kit at – 25° + 6° C.
			1. 5 freeze/thaw cycles are possible.
			2. Once Calibrator is prepared, **aliquot and freeze 450uL in the -70° freezer.** Aliquots should be prepared and labeled prior to being placed in the freezer. This will allow for more stability with the freeze and thaws.
		2. PCT Controls - Reconstitute with 2 ml of distilled water. Wait 5 -10 minutes and then mix. After reconstitution, the controls are stable for 8 hours at 2 to 8° C or until the expiration date of the kit at – 25° + 6 °C. 5 freeze/thaw cycles are possible.
			1. Once QC Material is prepared, **aliquot and freeze 250uL in the -70° freezer.** Aliquots should be prepared and labeled, prior to being placed in the freezer. This will allow for more stability with the freeze and thaws.
	2. Materials Required But Not Provided
		1. Calibrated pipette to dispense 2 mL and 200 μL.
		2. Distilled water
		3. Disposable pipette tips for pipette.
		4. Powder less disposable gloves.
		5. Mini Centrifuge Tubes
	3. Personal Protective Equipment
		1. Gloves
		2. Lab Coats

**4.0 SPECIMEN COLLECTION AND PREPARATION**

 4.1 Collect Human Serum or Plasma with Lithium Heparinate. For a given patient, the PCT

 assays must be performed on the same type of sample tube. **Since EDTA causes a decrease**

 in the values measured, plasma collected on EDTA should not be used (to test). Samples

 containing suspended fibrin particles or erythrocyte stroma should be centrifuged before

 testing.

 4.2 Sample preparation:

 4.2.1 Dry tubes: wait for samples to coagulate and centrifuge according to the tube

 manufacturer’s recommendations to eliminate fibrin.

 4.2.2 Frozen-stored samples: after thawing, all these samples must be clarified by

 centrifuging.

 4.3 The sera or plasma separated from the clot can be stored at 2-8°C in stoppered tubes for up to

 48 hours; if longer storage is required, freeze at -25 ± 6°C. Six-month storage of frozen

 samples does not affect the quality of results. Three freeze/thaw cycles were validated.

**5.0** **INTERFERENCES**

 5.1.None of the following factors have been found to significantly influence this assay:

 5.1.1 Hemolysis - after spiking samples with hemoglobin, up to 347 μmol/L

 (monomer).

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

 triglycerides.

 5.1.3. Bilirubinemia - after spiking samples with bilirubin, up to 547 μmol/L.

**However, it is recommended not to use samples which appear to be hemolyzed, lipemic or icteric and, if possible, to collect a new sample.**

**6.0 CALIBRATION**

5.1 VIDAS® PTC Protocol Data Entry

When using the assay for the first time and before reading the MLE data, scan the barcode (at the end of the package insert) using the instrument barcode reader. This reading will allow the VIDAS® PTC protocol data to be transferred to the instrument software for its update. These data should only be read the first time the assay is used.

5.2 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 a master lot entry (MLE) card included in each kit. If this operation is not performed before initiating the tests, the instrument will not be able to print results. The master lot entry (MLE) need 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.

 5.3 Calibration

Calibration, using the two calibrators provided in the kit, must be performed each time a new lot of reagents are opened, after the master lot data has been entered, and then every 28 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf life of the kit. The calibrators, identified by S1 and S2, must be tested in duplicate in the same run (see Operator’s Manual). The calibration values must be within the set RFV ("Relative Fluorescence Value"). If this is not the case, recalibrate using S1 and S2.

**7.0 NEW LOT CORRELATION**

7.1Due to possible manufacturing differences, new lots of Procalcitonin

reagent kits . must be tested and evaluated with the old lot prior to being used for patient testing to ensure consistent patient results.

7.2 Patient samples, previously tested, are saved and frozen in the -70°

freezer.

7.3 Form **VIDAS01-001 Form A** will be used to document lot to lots.

**8.0 QUALITY CONTROL**

 Two controls are included in each VIDAS B.R.A.H.M.S PCT 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 controls deviate from the expected values. Samples tested in the same run must be re-assayed.

**Note:** It is the responsibility of the user to perform Quality Control **every 24 hours.**

**9.0 EXPECTED VALUES FOR THE CONTROLS AND CALIBRATORS**

 The expected values for the controls and calibrators are printed on the MLE card. If the result from testing the controls and calibrators do not meet these specifications, do not report patient results.

**10.0 PROCEDURE**

 10.1. Remove the required reagents from the refrigerator.

 10.2. Use one "PCT" strip and one "PCT" SPR for each sample, control or calibrator to be

 tested. Make sure the storage pouch has been carefully resealed after the required SPRs

 have been removed.

 10.3. The test is identified by the "PCT" code on the instrument. The calibrators must be

 identified by "S1" and by “S2”, and tested in duplicate. If the controls need to be tested,

 they should be identified by C1 and C2 and tested singly.

 10.4. Mix the calibrators and/or controls using a vortex-type mixer.

 10.5. For this test, the calibrator, control, and sample test portion is 200 μl. Inoculate 200uL

 of patient specimen into the SPR in the front well. Check specimen prior to

 inoculating the SPR to ensure 200uL has been aspirated and no bubbles are present.

 Specimens with less than 200 uL should be canceled as QNS.

 10.6. Insert the "PCT" SPRs and strips into the appropriate position on the instrument.

 Check to make sure the color labels with the assay code on the SPRs and the Reagent

 Strips match.

 10.7. Initiate the assay immediately. All the assay steps are performed automatically by the

 instrument.

 10.8. Reclose the vials and return them to the required temperature after pipetting.

 10.9. The assay will be completed within approximately 20 minutes. After the assay is

 completed, remove the SPRs and strips from the instrument.

 10.10. Dispose of the used SPRs and strips into an appropriate recipient.

 10.11 Results from controls and patient tests can be searched and printed by clicking on the

 History tab and filling in the date range.

**11.0. RESULTS**

 Once the assay is completed, results are analyzed automatically by the computer using two calibration curves which are stored by the instrument; the concentrations are expressed in ng/mL.

With VIDAS PC, if a result which is < 0.05 ng/mL is obtained, the printed report will include the alarm “J2 > J4 & J2 – J0 < RFV threshold” and \*\*\* will be indicated for the RFV. This alarm, which is linked with the reading mode of the VIDAS B·R·A·H·M·S PCT (PCT) technique (dual reading), does not call into question the concentration measured. As no international standard is available, VIDAS® B·R·A·H·M·S PCT is calibrated against an internal panel of human sera with known procalcitonin concentrations. In case of patient follow-up, it is recommended to use the same PCT assay technique. Samples with procalcitonin concentrations greater than 200 ng/mL should be retested after dilution by 1/10 (1 volume of sample + 9 volumes of PCT negative sample).

**Interpretation of PCT values in critically ill ICU Patients.**

SIRS, sepsis, severe sepsis, and septic shock are categorized according to the criteria of the consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine.

< 0.5 ng/mL Systemic infection (sepsis) is not likely. Local bacterial infection is possible.

≥0.5 and < 2 ng/mL Moderate risk for progression to severe systemic infection (severe sepsis). The patient should be closely monitored both clinically and by re-assessing PCT within 6-24 hours.

≥ 2 and < 10 ng/mL High risk for progression to severe systemic infection (severe sepsis).

≥10 ng/mL Important systemic inflammatory response, almost exclusively due to severe bacterial sepsis or septic shock. High likelihood of severe sepsis or septic shock.

**Interpretation of PCT values for differential diagnosis of Lower Respiratory Tract Infections.**

<0.1 ng/mL Indicates absence of bacterial infection.

≥ 0.1 and <0.25 ng/mL Bacterial infection unlikely.

≥ 0.25 and <0.5 ng/mL Bacterial infection is possible.

≥0.5 ng/mL Suggests the presence of bacterial infection.

Patient results should be recorded on Form **VIDAS01-01 Form A.**

**12.0 MAINTENANCE**

 12.1 Monthly: Clean the SPR Blocks

 12.2 Semi-Annually or As Needed

 12.2.1 Clean Housing and Front Cover

 12.2.2 Clean Vials, Tubes & Disposables Rack\*

 12.2.3 Clean Waste Drawer\*

 12.2.4 Clean Reagent Strip Sections\*

 12.2.5 Clean Touch Screen

Maintenance should be recorded on **VIDAS01-001 Form C Maintenance Checklist.**

**13.0 LIMITATIONS OF PROCEDURE:**

 Interference may be encountered with certain samples containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history and the results of any other tests performed.

**14.0 REFERENCE:**

 VIDAS® B•R•A•H•M•S PCT (PCT) package insert. Refer to the insert for the complete details of the procedure, references, and performance of the product.

Approval Signatures:

|  |  |  |
| --- | --- | --- |
| Date | Printed Name | Signature |
| 5/27/2016 | Jennifer Lore, MFS, MTChemistry Supervisor |  |
| 5/27/2016 | Nancy A. Young, M.D., FCAPMedical Director |  |

**Review History**

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