VIDAS 3 (PCT) PROCALCITONIN

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PURPOSE

To provide instructions for the quantitative determination of procalcitonin on the Biomerieux Vidas 3.

PRINCIPLE

Procalcitonin test reaction strips, when used in conjunction with Vidas/MiniVidas System(s) VIDAS PCT calibrator, are intended for the quantitative determination of Procalcitonin concentration in human plasma.

BACKGROUND

Clinical Significance

Procalcitonin is the prohormone of calcitonin that is secreted by different types of cells from numerous organs in response to pro-inflammatory stimulation. In patients with bacterial infection the PCT levels increase rapidly. This distinction enables PCT to be a diagnostic marker in differentiating bacterial sepsis from other septic causes. Sepsis is an excessive reaction of the immune system and coagulation system to an infection. Viral infections, allergies, autoimmune diseases and graft rejection do not lead to a significant increase in PCT. Additionally, reabsorption of bacterial septic infection is accompanied by a decrease in the PCT concentration which returns to normal with a half-life of 24 hours. In certain situations (newborns, polytrauma, burns, major surgery, prolonged or severe cardiogenic shock, etc.) PCT elevation may be independent of any infectious aggression. The return to normal values is usually rapid.

Methodology

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 prepared and are pre-dispensed in the sealed Reagent Strips. All of the assay steps are performed automatically by the Vidas/mini Vidas. 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 immunoglobulin fixed to the interior wall of the SPR and the conjugate to form a sandwich. Unbound compounds are eliminated during the washing steps. Two detection steps are performed successively. During each detection step, the substrate (4-methyl-umbellilaryl 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.

RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
M-F-CH-0820	Chemistry Controls
M-F-CH-0826	Chemistry Calibrators

SPECIMEN

Type of Specimen

The preferred sample is Plasma collected in Li Heparin. Serum is also acceptable. For a given patient, the PCT assays must be performed on the same type of sample tube.

Specimen Storage and Stability

1. Plasma must be collected in Li Heparin. Plasma or serum must be free of suspended fibrin and/or erythrocyte stroma. Re-centrifuge plasma if necessary.

2. Separated samples can be stored at 2-8 °C for up to 48 hours.

3. Plasma or serum can be left at room temperature for up to 4 hours, but it is recommended to refrigerate as soon as possible.

4. Fresh or frozen plasma or serum can be used.

5. Frozen samples at –25 +/- 6 °C are good for up to 6 months. Three freeze/thaw cycles have been validated.

6. After thawing, previously frozen samples must be clarified by centrifugation.

Sample Type	Volume	Sample Stability
LithiumHep Plasma/ Serum	200uL	• 4 hours at 18-26°C, refrigerate sooner if possible • 48 hours at 2-8°C • After 48 hours, freeze at –25 +/- 6 °C • Frozen plasma, good for up to 6 months • 3 freeze and thaw cycles have been validated • After thawing, previously frozen samples must be clarified by centrifugation.

Sample Preparation

Plasma or serum must be free of suspended fibrin and/or erythrocyte stroma. Re-centrifuge plasma or serum if necessary.
Re-centrifuge thawed samples.

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

Plasma/Serum Volume per Test	
Sample Volume	200uL

REAGENTS

Contents

Vidas Brahms Procalcitonin (PCT) kit, Reference 30450-01.

Contents of the 60-test kit:

- 60 PCT ready to use Reagent Strips
- 60 (2x30) PCT ready to use Solid Phase Receptacles (SPR's). Interior of SPR's are coated with mouse monoclonal anti-procalcitonin immunoglobulins.
  NOTE: Carefully reseal the pouch with the dessicant inside after use to maintain stability of the SPR's. Before use, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPR's.
- PCT lyophilized controls (white caps), 2 vials of C1 and 2 vials of C2. Controls are specific to kit lot #.
- PCT lyophilized calibrators/standards (red caps), 2 vials of S1 and 2 vials of S2. Standards are specific to kit lot #.

No warm-up time is required for reagents or SPR's.

Description of the PCT reagent strip:

Wells	Reagents
1	Sample well
2, 3, 4	Empty wells
5	Conjugate: alkaline phosphatase-labeled mouse monoclonal anti-human procalcitonin immunoglobulins + preservative (400 µL).
6, 7, 8	TRIS NaCl Tween (pH 7.3) + preservative (600 µL).
9	Empty well
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine* (DEA*) (0.62 mol/L or 6.6%, pH 9.2) + 1g/L sodium azide (300 µL).

For complete instructions see the VIDAS/mini VIDAS Operator's Manual.

Reagent Preparation

Ready to use.

Acceptable Reagent Performance

The acceptability of a reagent kit is determined by successful calibration and by ensuring that quality control results are within your facility`s acceptance criteria.

Reagent Storage and Stability

Vidas Brahms Procalcitonin (PCT) Reagents strips and SPR's, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on them. Do not use beyond the manufacturers expiration date.

CALIBRATION

Calibrators Required

1. PCT lyophilized calibrators, 2 vials of S1 and 2 vials of S2 per kit
2. Calibrators S1 and S2 are tested in duplicate in the same run, every 28 days.
3. Calibrators are specific to kit lot #.
4. 1 MLE barcode on side of kit box– has the acceptable limits for calibrators and QC.

Calibrator Preparation

1. Reconstitute S1 and/or S2 with 2 ml of DI H2O.
2. Let stand 5-10 minutes then mix.
3. Mix the controls using a vortex-type mixer.
4. Stable after reconstitution for 8 hours at +2°C to +8°C or until the expiration date on the kit at –25 +/- 6 °C.
5. Five freeze/thaw cycles are allowed.
6. Calibrators can be frozen in 500 ul aliquots.
7. Note aliquot date, expiration date and kit lot# on aliquots.

Calibrator Storage and Stability

If unopened, Vidas Brahms Procalcitonin (PCT) Calibrators, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on them. Do not use beyond the manufacturer's expiration date. After reconstitution, calibrators are stable for 8 hours at 2-8°C or until the expiration of the kit at -25 ± 6°C. 5 freeze/thaw cycles are possible.

New Lot Information

Before each new lot of reagents is used, specifications (or factory master calibration curve data) must be entered into the instrument. If this operation is not performed before initiating the tests, the instrument will not be able to print results. The master lot data need only be entered once for each lot.

The MLE information can be entered in using the barcode on the outside of the kit box.

- Select the Calibration icon from the Navigation Toolbar
- Select the MLE icon in the Action Toolbar and the Scan MLE Barcode screen appears.
- Slowly scan the barcode on the outside of the kit box until you hear a beep.
- The Master Lot information will be loaded into the analyzer.

Calibration Information

Calibration, using the two calibrators provided in the kit, must be performed, in duplicate, within the same run:

- each time a new lot of reagent is opened,
- after the master lot data (MLE) has been entered
- every 28 days.
- if QC data indicates calibration is needed.

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 (see VIDAS Operator's Manual) in the same run. The calibration values must be within the set RFV ("Relative Fluorescence Value"). If this is not the case, recalibrate using S1 and S2.

Performing Calibration

1. Take out 6 SPR's and 6 Reagent Strips

2. Thaw or make up enough standards and controls to run 2 tests each for the standards and one test each for the controls.

3. Program the Calibration by selecting the ANALYSES Icon from the Navigation Toolbar.

4. Select the + icon next to CALIBRATION and the calibration entry screen will appear. Select PCT assay, Lot number (from dropdown menu), and select automatic or manual pipetting mode. Click SAVE and CLOSE.

5. The calibration test requests will appear in the calibration section of the analyses screen. Highlight PCT on the Calibration line and click LOAD from the Action Toolbar.

6. When standards and controls are pipetted and SPR's and Reagent Strips are in place, choose START.

7. Print the calibration details report upon completion. Write the calibrator open expiration date and your Tech ID on the calibration printout and save it in the designated place for your lab.

QUALITY CONTROL

See Related Document M-F-CH-0820 Chemistry Controls

Controls Required

1. PCT lyophilized controls, 2 vials of C1 and 2 vials of C2 per kit

2. Controls C1 and C2 are tested
   - each day of use
   - immediately after monthly maintenance
   - Immediately after calibration
   - When starting a new kit lot or shipment

3. Controls are specific to kit lot #.

4. Control ranges are programmed in the MLE data. They can be viewed by clicking on the CALIBRATION icon from the Navigation Toolbar, then highlight the current calibration and select DETAILS from the Action Toolbar. Control ranges listed on the MLE printout are 3SD ranges and we need to use 2SD ranges. Refer to LIS for correct QC ranges (or manually written ranges if LIS is not updated for a new QC lot number).

Control Preparation

1. Reconstitute with 2 ml of DI H2O.
2. Let stand 5-10 minutes then mix.
3. Mix the controls using a vortex-type mixer.
4. Stable after reconstitution for 8 hours at +2°C to +8°C or until the expiration date on the kit at –25 +/- 6 °C. .
5. Five freeze/thaw cycles are possible.
6. Freeze controls in 1 mL aliquots.
7. Note aliquot date, expiration date and kit lot#, control level (C1 or C2) on aliquots.
8. Patient results should not be validated if the control values deviate from the expected values.

STEPS

NOTE: if this is the first time a new lot number is being used, the MLE information must be read into the Vidas 3. Refer to New Lot Information section of this document or the Operator's manual for this process.

1. Remove the required PCT strips and PCT SPRs from the refrigerated kit. **Make sure the storage pouch has been resealed with the desiccant pouch inside, after the required SPRs have been removed.**
2. Take out and thaw the required controls (if more than 24 hours since last control run) and/or calibrators (if more than 28 days since last calibration) from the freezer. Product may be aliquoted. Reconstitute new controls and calibrators if necessary.

   If more than 28 days from the last calibration, run calibration with S1 and S2 in duplicate in the same run.

   If more than 24 hours since last QC run, run C1 and C2 controls in singlet. Controls can only be evaluated by the instrument if they are programmed as C1 and C2 and loaded correctly. Control ranges programmed in the instrument MLE data are 3SD ranges and we need to use 2SD ranges. Refer to LIS for correct QC ranges (or manually written ranges if LIS is not updated for a new QC lot number).
3. Mix calibrators and controls before running. Ensure that samples, calibrators and controls are free of bubbles.
4. **To program Calibration run**: see Performing Calibration section.

   To program Control run: select the ANALYSES menu from the Navigation Toolbar. Select the + icon next to KIT CONTROL and the Control entry screen will appear. Select PCT assay, Lot number (from dropdown menu), levels and select automatic or manual pipetting mode. Click SAVE and CLOSE. The control test requests will appear in the kit control section of the analyses screen. Highlight PCT on the kit control line and click LOAD from the Action Toolbar.

   For **Automated** control pipetting by the Vidas 3:
   - Check barcoded control vials for bubbles and ensure appropriate volume is available for automated pipetting. If volume is low, see Manual pipetting.
   - Remove the sample rack completely from the Vidas instrument.

- Remove caps and stoppers from control vials and load control vials into sample rack in the purple section with the barcode(s) facing out.
- Reinsert the rack horizontally and smoothly.

For **Manual** control pipetting:

- Change pipetting mode to Manual (either edit from the control tests requests screen or by highlighting the programmed control from the LOADING screen and selecting the Manual button on the Action toolbar)
- Pipet 200 uL of control into the first well of the reagent strip, avoiding bubbles.

To program Patient run:

1. Select the ANALYSES menu from the Navigation Toolbar.
2. Click CREATE.
3. Scan the sample barcode into the Sample ID field.
4. Enter the patient first and last name into the Patient Name field.
5. Select PCT assay
6. If necessary, click Edit to choose "Emergency" (stat) or to change pipetting mode to Manual.
7. Click SAVE and CLOSE.
8. Highlight the patient line on the analyses screen and click LOAD from the Action Toolbar.

For **Automated** sample pipetting by the Vidas 3:

- A minimum of **1 mL** plasma/serum must be present in the tube (if <1 mL, see Manual Pipetting)
- Remove cap from sample and ensure the sample is free of bubbles.
- Completely remove sample rack and load sample tube into sample rack in the blue section with the barcode facing out.
- Reinsert the rack horizontally and smoothly into the instrument.

For **Manual** sample pipetting:

- Change pipetting mode to Manual (either edit from the Create request screen or by highlighting the programmed sample from the LOADING screen and selecting the Manual button on the Action Toolbar).
- Pipet 200 uL of patient sample into the first well of the reagent strip, avoiding bubbles.

5. Insert the "PCT" SPR and Strip into the appropriate position in the instrument. Check that both the SPR and Reagent Strip are in the corresponding positions.

6. Initiate the assay immediately by pressing "START". The instrument performs all the assay steps automatically. The assay will be completed in approximately 20 minutes. Ensure that the status lights and indicators indicate that the run has begun.

7. The Vidas 3 will notify the user to remove control vials immediately after sampling if loaded in Automated pipetting mode. Return control vials to freezer immediately.

8. At the completion of the assay, the status on the LOADING screen will say "UNLOAD" for the completed section, and the green light will blink. Discard the SPR's and Reagent Strips into a biohazard waste container.

9. Print results. Write the patient's last and first name next to their accession number (or affix an LIS label with the patient's name) if patient name is not present on printout.

10. Record your tech ID on the printout.

11. Enter the control and patient results into the LIS and place the printouts in the appropriate place for your site.

CALCULATIONS

Vidas 3 System performs all calculations internally to produce the final reported result.

RESULT INTERPRETATION

Infections (excluding LOWER RESPIRATORY TRACT)	
PCT Concentration	**Interpretation**
<0.05	Negative/Normal
0.05-0.49 ng/ml	Sepsis unlikely. Local bacterial infection possible. Low risk for progression to severe sepsis/septic shock.
0.50 - 2.00 ng/ml	Sepsis possible. Moderate risk for progression to severe sepsis/septic shock.
2.01 -9.99 ng/ml	Sepsis is likely. High risk for progression to severe sepsis/septic shock.
≥10 ng/ml	High likelihood of severe sepsis or septic shock.
NOTES: Clinical correlation is required. It is recommended that an initial PCT concentration of <2 ng/ml be retested within 6-24 hours if clinical suspicion of sepsis exists. PCT repeat testing should be determined by the patient's physician and pharmacist to determine optimal follow-up. Neonates <48 hours old have increased PCT values without corresponding sepsis. Grossly hemolyzed samples should be rejected and recollection requested.	

LOWER RESPIRATORY TRACT INFECTIONS	
PCT Concentration	**Interpretation**
<0.10	Indicates absence of bacterial infection. Use of antibiotics strongly discouraged, even in the presence of impaired pulmonary reserve in AECOP.
0.10 - 0.24 ng/ml	Bacterial infection unlikely. Use of antibiotics is discouraged.
0.25 - 0.49 ng/ml	Bacterial infection is possible. Recommend to initiate antimicrobial therapy.
≥ 0.50 ng/ml	Suggests the presence of bacterial infection. Antibiotic treatment strongly recommended.
NOTES: Clinical correlation is required. It is recommended that an initial PCT concentration of <2 ng/ml be retested within 6-24 hours if clinical suspicion of sepsis exists. PCT repeat testing should be determined by the patient's physician and pharmacist to determine optimal follow-up. Neonates <48 hours old have increased PCT values without corresponding sepsis. Grossly hemolyzed samples should be rejected and recollection requested.	

ANTICOAGULANT TEST RESULTS

Lithium Heparin is the only acceptable anticoagulant.

ANALYTIC RANGE

Sample Type	Conventional Units
Plasma	0.05- 150 ng/mL

Reporting results outside of analytical range

Lower limit of linearity	0.05 ng/ml	Results less than 0.05 should be reported as “<0.05 ng/mL”
Upper limit of linearity	150 ng/ml	Results greater than 150 should be reported as “>150 ng/mL”

LIMITATIONS

In certain situations (newborns, polytrauma, burns, major surgery, prolonged or severe cardiogenic shock, etc.) PCT elevation may be independent of any infectious aggression.

Interferences

Interference may be encountered with certain antibodies directed against reagent components.

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

Substance	Level Tested
Hemoglobin	600 mg/dL
Lipemia- Triglycerides	3000 mg/dL
Bilirubin	33 mg/dL

Grossly hemolyzed samples should be rejected and recollection requested.

Specificity

The following compounds, tested at the concentrations indicated in the table, do not affect the VIDAS B·R·A·H·M·S PCT test.

Tested compound	Tested concentration
Protein (albumin)	6.5 g/dL
Human Calcitonin	60 ng/mL
Human Katacalcin	10 ng/mL
Human a-CGRP*	10 µg/mL
Human b-CGRP*	10 µg/mL

*Calcitonin Gene Related Peptide

Drug Interference

Following the recommendations of CLSI® document EP7-A2, the potential interferences with the following drugs and potentially interfering substances were studied. No interference was observed at the concentration tested.

Tested Drug	Tested Concentration
Acetominophen (paracetamol)	20 mg/dL
Acetylsalicylic Acid	65.2 mg/dL
Alcohol	400 mg/dL
Amoxicillin	7.53 mg/dL
Ampicillin	5.31 mg/dL
Azithromycin	1.15 mg/dL
Beclometasone dipropionate	0.1 mg/dL
Caffeine	5.98 mg/dL
Cefotaxime	32.13 mg/dL
Ceftriaxone	93.7 mg/dL
Celecoxib	24 mg/dL
Cetirizine HCl	0.36 mg/dL
Cromolyn	2.4 mg/dL
Dextromethorphan	0.14 mg/dL
Dobutamine	0.15 mg/dL
Dopamine	0.11 mg/dL
Epinephrine (adrenaline)	0.18 mg/dL
Fluticasone	0.03 mg/dL

Tested Drug	Tested Concentration
Formoterol	0.0029 mg/dL
Furosemide	5.98 mg/dL
Heparin sodium	3000 UI/L
Ibuprofen	50 mg/dL
Imipenem	18 mg/dL
Levoflaxacin	1.75 mg/dL
Linezolid	48 mg/dL
Loratadine	0.03 mg/dL
Naproxen	50 mg/dL
Nicotine	0.1 mg/dL
Noradrenaline	0.00021 mg/dL
Oxymetazoline HCl	0.009 mg/dL
Phenylephrine	0.018 mg/dL
Prednisolone	0.3 mg/dL
Salmeterol	0.006 mg/dL
Theophylline	4 mg/dL
Tiotropium	0.0022 mg/dL
Vancomycin	10.25 mg/dL

ADDITIONAL INFORMATION

For more detailed information on Biomerieux Vidas 3 System, refer to the appropriate system manual.

REFERENCES

Biomerieux Vidas B R A H M S PCT package insert, revision 02/2017
Vidas 3 User Manual 161150-465 A 06/2015