MINIVIDAS (PCT) PROCALCITONIN

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PURPOSE

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

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. Viral infections, allergies, autoimmune diseases and graft rejection do not lead to a significant increase in PCT.

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-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
M-F-CH0820	Chemistry Controls
M-F-CH0826	Chemistry Calibrators

SPECIMEN

Type of Specimen

The preferred sample is Plasma collected in Li Heparin. Serum is also acceptable.

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.
- 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 #.
- 1 MLE card - specification sheet containing the master calibration data required to calibrate the test.

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

Allow reagent strip and SPR to come to room temp.

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 card - specification sheet containing the master calibration data is required to calibrate the test. Concentration in ng/ml is listed on the MLE card.

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. Freeze calibrators in 500 ul aliquots so two reagent strips can be innoculated.

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.

Calibrator Information

Before each new lot of reagents is used, specifications (or factory master calibration curve data) must be entered into the instrument using the master lot entry (MLE) card (specifications sheet) 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 data need only be entered once for each lot.

The information from the MLE card can be entered in using the barcode on the outside of the kit box.
- From the MAIN MENU, select "MASTER LOT MENU"
- Select "Scan Master Lot".
- Scan the barcode on the outside of the kit box.

Or it is possible to enter data using the MLE card.
- Insert the MLE card, face up, on a tray holder (found in drawer).
- From the MAIN MENU, select "MASTER LOT MENU".
- Select section A or B.
- Wait for completion, approximately 2 minutes.

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 card) 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 test each for the standards and one test each for the controls. Select STATUS SCREEN, section A or B,

3. Program in the standards and controls:
   - Choose position 1, "S", 1, enter;
   - Then 2, "S", 1, enter, and so on until each standard is entered twice and each Control once.

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

5. Write the calibrator open expiration date on the calibration printout and save it in the designated place for your lab.

6. Write the date on the calibration card posted on the instrument.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 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 calibration
   - When starting a new kit lot or shipment

3. Controls are specific to kit lot #.

4. Control ranges listed on the MLE card 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 300 ul aliquots.

7. Note aliquot date, expiration date and kit lot# 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 card information must be read into the miniVidas. This can be done in more than one way. Refer to Calibration 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 is usually 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. The instrument evaluates based on a 3SD range. Refer to LIS for correct QC ranges (or manually written ranges if LIS is not updated for a new QC lot number).

3. Mix (vortex) calibrators and controls before running.

4. Pipette 200 ul of calibrator, control, or patient sample into the reagent strip sample well.

5. Load the SPR into the SPR compartment and the Reagent Strip into the tray. Check that both the SPR and Reagent Strip are in the corresponding positions.

   NOTE: THE INSTRUMENT DOES NOT CHECK FOR SPR'S.

6. To preprogram the run, select the "STATUS SCREEN" key. Select section A or B. Press the number for the position to be used. Select either S for standard, C for control or Sample ID for patient. Enter the sample number via the keyboard or barcode reader. Press Enter, if necessary to move to the next position.

7. 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 "Green Light" is "on" before leaving the instrument.

8. At the completion of the assay, the STATUS 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. **VERIFY SPR'S WERE LOADED! IF SPRs ARE NOT LOADED, YOU WILL GET ERRONEOUS RESULTS, NOT AN ERROR CODE.**

10. Write the patient's last and first name next to their accession number (or affix an LIS label with the patient's name).

11. Record your tech ID on the printout.

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

CALCULATIONS

Mini Vidas System performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

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
Hemolysis	up to 347 umol/L
Lipemia	up to 30 g/L equivalent in triglycerides
Bilirubinemia	up to 574 umol/L.

Specificity

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

Tested compound	Tested concentration
Protein (albumin)	4 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

The following drugs, at the concentrations indicated in the table, do not affect the mini VIDAS B·R·A·H·M·S PCT test:

Tested Drug	Tested concentration
Imipenem	0.5 mg/mL
Cefotaxime	180 mg/dL
Vancomycin	3 mg/mL
Dopamine	26 mg/dL
Noradrenalin	4 µg/mL
Dobutamine	22.4 µg/mL
Heparin	16,000 U/L
Furosemide	4 mg/dL

ADDITIONAL INFORMATION

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

REFERENCES

Biomerieux Vidas B R A H M S PCT package insert, revision 09/2010

DOCUMENT APPROVAL Purpose of Document / Reason for Change:			
Changing upper end of the reportable range from 200 to 150 ng/mL.			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☒ Date: 7/23/15 ☐ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 7/27/15