TRAINING UPDATE

Lab Location:	SGMC & WOMC	**Date Distributed:**	8/19/2020
Department:	Core Lab	**Due Date:**	9/2/2020
		Implementation:	**9/2/2020**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Procalcitonin Test by Biomerieux Vidas 3 SGAH.C975 v2

Description of change(s):

Note: The major changes include transmitting QC to Bio-Rad electronically, auto-verification / transmission of patient results and handling dilutions in DI.

Section	Reason
Header	Changed WAH to WOMC
6.3	Added steps to transmit QC
8.4	Added processing dilutions in DI< auto-verification of results & reference to add. B
10.5	Deleted manual resulting
17	Add QC information
19	Add addendum B

This revised SOP will be implemented on September 2, 2020

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Procalcitonin Test by Biomerieux Vidas 3**
Prepared by	Julie Negado and Zanetta Morrow Date: 8/28/2017
Owner	Robert SanLuis Date: 8/28/2017

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Procalcitonin	Enzyme-Linked Fluorescent Assay / Vidas 3	PCT

Synonyms/Abbreviations
Procalcitonin, PCT

Department
Chemistry

2. ANALYTICAL 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 wash steps.

Two detection steps are performed successively. During each step, the substrate (4-Methy-umbulliferyl 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.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Not applicable

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) N/A	
Collection Container	Mint green top tube (PST)	
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL	
Transport Container and Temperature	Collection container at room temperature	
Stability & Storage Requirements	Room Temperature:	8 hours
	Refrigerated:	48 hours
	Frozen:	6 months, do not exceed three (3) freeze/thaw cycles
Timing Considerations	Specimen should be tested as soon as possible.	
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.	
Compromising Physical Characteristics	Hemolyzed, lipemic and icteric samples reject sample and request a recollection. Credit the test with the appropriate LIS English text code.	
Other Considerations	None	

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Vidas B.R.A.H.M.S PCT Kit (include PCT Reagent Strips, SPRs, Calibrators and Controls)	BIOMERIEUX Ref. # 30-450-01
NERL Reagent Grade Water	Thermo Scientific NERL® Reagent Grade Water Ref.#9800-3

4.2 Reagent Preparation and Storage

Reagent	**PCT Reagent Strips, PCT SPRs**
Storage	2-8°C
Stability	Stable until kit expiration date
Preparation	Ready to use

Reagent	**NERL Reagent Grade Water**
Storage	Store at room temperature.
Stability	Stable for 30 days after opening.
Preparation	None

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
PCT Calibrators (S1 and S2) (included in the PCT Kit)	BIOMERIEUX Ref. # 30-450-01

5.2 Calibrator Preparation and Storage

Reagent	**PCT Calibrators (S1 and S2)**
Storage	Store at 2-8°C
Stability	Once reconstituted, the calibrators are stable for 8 hours at 2-8°C, or until expiration date on the kit at -25+/- 6°C. (Freeze immediately after reconstitution preferably in aliquots of 200 µL) Five (5) freeze and thaw cycles are possible.
Preparation	Reconstitute with 2 mL distilled water. Let stand for 5-10 minutes then mix.

5.3 Calibration Criteria and Procedure

Criteria	Special Notations
Frequency	Performed each time a new lot of reagent is opened, after the master lot data (MLE) has been entered and then every 28 days.
Tolerance Limits	The calibration values must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate using S1 and S2)
Procedure	• Calibration is performed by Testing Personnel • Remove the required reagents from the refrigerator. • Use one "PCT" strip and one "PCT" SPR for each calibrator to be tested. Ensure the storage pouch has been carefully

	resealed after the required SPRs have been removed. • The calibrators must be identified by "S1" and by "S2" and tested in duplicate. • Mix the calibrators using a vortex-type mixer after reconstitution. • Follow steps for Test Run in section 8.3. • At the completion of the calibration, results are analyzed automatically by the computer using two calibration curves. The calibration values must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate using S1 and S2.
Dilutions	N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Vidas B.R.A.H.M.S PCT Controls C1 and C2 (included in the PCT Kit)	BIOMERIEUX Ref. # 30-450-01
Lyphocheck Specialty Immunoassay Controls L1 and L2	Bio-Rad Laboratories Ref.#27124 and 27125

6.2 Control Preparation and Storage

Reagent	**PCT Controls (C1 and C2)**
Storage	Store at 2-8°C
Stability	Once reconstituted, the controls are stable for 8 hours at 2-8°C, or until expiration date on the kit at -25+/- 6°C. (Freeze immediately after reconstitution preferably in aliquots of 200 µL) Five (5) freeze and thaw cycles are possible
Preparation	Reconstitute with 2 mL distilled water. Let stand for 5-10 minutes then mix with vortex

Control	**Lyphocheck Specialty Immunoassay Controls L1 and L2**
Storage	Store at 2-8°C
Stability	**Unopened**: until expiration date when stored at 2-8°C. **Reconstituted and stored tightly capped at 2-8°C**: 3 days. **Reconstituted and stored in tightly capped aliquot vials at -20 to -70°C**: stable for 30 days. Use the content of each aliquot vial only once and discard the remainder.

Preparation	Reconstitute each vial with 2 mL of distilled or deionized water. Replace the stopper and allow this product to stand for approximately 15 minutes swirling occasionally. Before sampling, gently swirl the vials several times to ensure homogeneity.

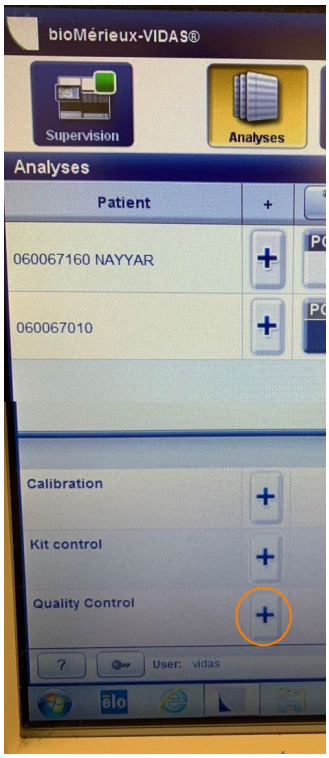
6.3 Frequency

Vidas B.R.A.H.M.S PCT Controls C1 and C2 must be performed immediately after opening a new kit to ensure that the reagent performance has not been altered. Each calibration must also be checked using these controls.

Specialty Immunoassay Controls L1 and L2 will be run after opening a new Vidas B.R.A.H.M.S PCT reagent kit, after a calibration and once per shift each day of patient testing.

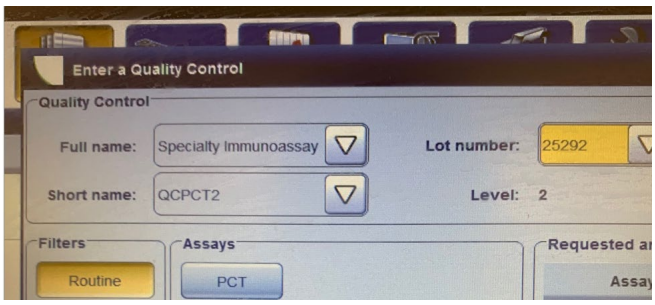
Programming QC on the Vidas

a. From the main screen click on the + **sign** next to Quality Control

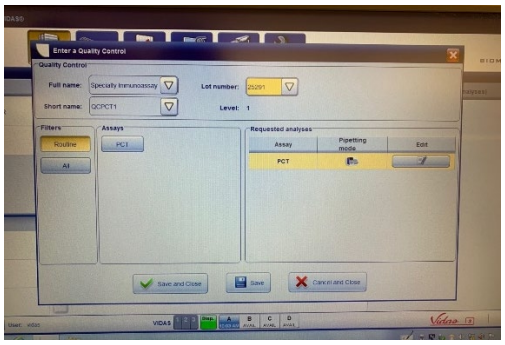


b. To program the QC, select preprogramed names from the drop down.
Full Name: Specialty Immunoassay
Short Name:
SGMC: QCPCT1 for level 1
QCPCT2 for level 2
WOMC: PCTQC1 for level 1
PCTQC2 for level 2

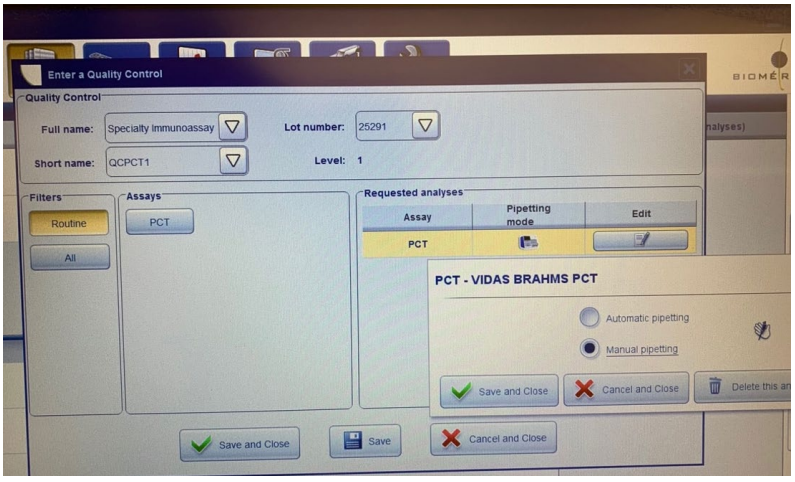
Select the lot number



c. Click on PCT Assay. PCT displays under Requested analyses. Click on **Edit**

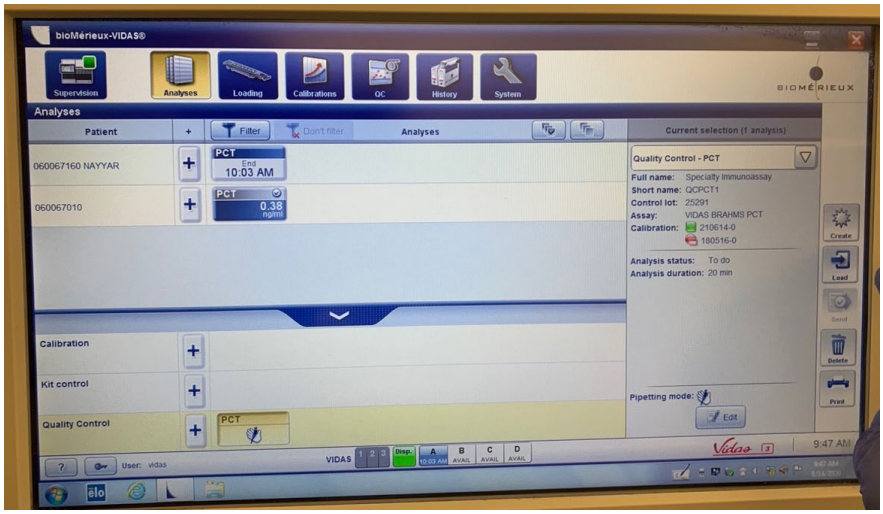


d. Window opens. Select **Manual pipetting** then click on **Save and Close**. Then click on **Save and Close** again.
e. Pipette QC level into test strip.



f. Repeat steps a through e for second level of QC.

g. From the main screen. Proceed to load the Test Strip.
h. Click PCT icon in QC section.
- Current selection information displays on the right side.
- The short name will tell you what QC level to run.
- Next click on **Load**.
- Insert the test strip into the position indicated on the screen.
- Click on **Start**.



QC results will transmit to Bio-Rad.

Note: If QC results do not transmit, follow steps below to manually enter into Bio-Rad.

1. Log into Unity Real Time
2. Select the appropriate Lab site
   a. WOMC: ~~"216442 WAH Centaur"~~ "221165: WOMC Vidas 1"
   b. SGMC: ~~"137244 SGAH Centaur"~~ "221161: SGAH Vidas"
3. Select Specialty Immunoassay
4. Control 1 results are entered as Level 1
5. Control 2 results are entered as Level 2
6. SAVE

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and

Step	Action
	patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented appropriately in Bio Rad Unity. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult and follow corrective action guidelines in Laboratory QC Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time where the cumulative mean, SD, and CV are all calculated and stored for easy retrieval.
- Quality Control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for records retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot of reagent or new shipment of the same lot of reagent must be tested with external QC control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEa for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

VIDAS® B.R.A.H.M.S PCT ™

7.2 Equipment

Biomerieux Vidas 3

7.3 Supplies

Pipettes to dispense 2 mL and 200uL
Pipette tips
PPE defined by lab

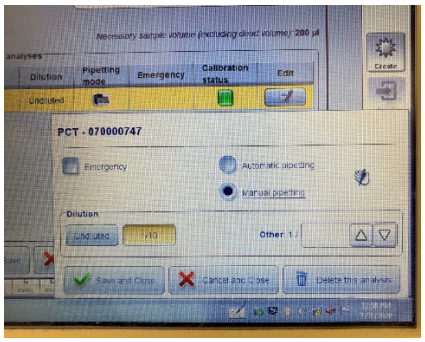
8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Maintenance
1.	Perform required maintenance. Refer to addendum A for details

8.2	Specimen / Reagent Preparation
1.	Remove the required reagents from the refrigerator.
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 SPR®s has been removed.

8.3	Test Run
1.	Before pipetting, ensure that samples, calibrators and controls are free of bubbles.
2.	Mix the calibrators and controls with a vortex-type mixer in order to improve result reproducibility.
3.	Pipette 200µL of sample, calibrator or control into the sample well. (NOTE: the calibrators must be identified by "S1" and by "S2", and tested in duplicate. The controls, identified by C1 and C2, will be tested in singles.
4.	For patient testing, scan the Sunquest accession number. Insert the VIDAS SPRs and strips into the positions indicated on the screen. Verify that the color labels with the assay code on the SPRs and strips match.
5.	~~Initiate the assay processing as directed in the Operator's Manual~~ Click the blue start arrow. The instrument performs all the assay steps automatically. The assay will be completed in approximately 20 minutes.

8.3	Test Run
6.	In DI-Instrument Manager SM workspace, if the result has a > sign, you will need to: a. Dilute sample with 1:10 dilution (refer to section 10.5 for details). b. Scan the Sunquest accession number and program the dilution factor on the instrument. **Note**: Do NOT add extra characters to the SQ accession number (we will not be able to track the history in DI-Instrument Manager).  c. Rerun.
7.	After the assay is completed, dispose of SPRs and strips into bio hazardous waste containers.
8.	~~Print the Report and manually enter results into Sunquest under function MEM.~~ When testing is completed, results will automatically transmit to DI-Instrument Manager. If results are within the reference range, they will auto-verify in DI-Instrument Manager and then be sent to Sunquest. The results will flow through the SM workspaces associated with CHEM in DI-Instrument Manager for SGMC and WOMC.
9.	See addendum B for steps to resend results from the Vidas to DI-Instrument Manager

8.4	Special Handling
1.	To load a barcoded sample, remove the appropriate rack completely from the VIDAS 3 instrument.
2.	Load the sample into the appropriate segment of the rack.
3.	Reinsert the rack horizontally into the instrument. VIDAS 3 will automatically read the barcodes and update the Sample/Reagent Loading Plan.
4.	Load strips and SPR's according to the loading plan
5.	Ensure the disposables are available and select Start to begin analysis

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates the concentration of Procalcitonin in ng/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

No rounding necessary. Instrument reports results with two decimal points.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.05 – 2000.00 ng/mL

10.5 Repeat Criteria and Resulting

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 or Serum free reagent (Ref. 66 581). If the dilution factor has not been entered when the analysis has been requested (see Operator's Manual), multiply the result by the dilution factor to obtain the sample concentration.

IF the result is …	THEN…
< 0.05 ng/mL	Assure there is sufficient sample devoid of cellular debris, and/or fibrin clots. Report as: "< 0.05 ng/mL"
>200 ng/mL	Manually dilute the sample using dilution factor of 10. [1 volume of sample + 9 volumes of PCT negative sample (<0.5ng/mL)] **Diluent:** PCT negative sample. Enter the dilution factor on the instrument. The instrument calculates automatically (to program a dilution refer to the Vidas 3 manual).
>2000 ng/mL (after dilution)	If the recommended dilution does not give results within the clinically reportable range, report as: "> 2000 ng/mL".

~~**LIS Reporting**~~

~~Use LIS function MEM to enter results~~

11. EXPECTED VALUES

11.1 Reference Ranges

<0.10 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Sepsis is a daily challenge in intensive care units. There are various known therapeutic strategies to improve survival in patients with sepsis. Early assessment is important for determination of the appropriate treatment. PCT is the prohormone of the hormone calcitonin, but PCT and calcitonin are distinct proteins. Calcitonin is exclusively produced by C-cells of the thyroid gland in response to hormonal stimuli, whereas PCT can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products.

In healthy people, plasma PCT concentrations are found to be below 0.1 ng/mL. PCT level rises rapidly within 6 to 12 hours after a bacterial infectious insult with systemic consequences. Early onset of multiple traumas, major surgery, severe burns, or in neonates, PCT levels can be elevated independently of an infectious process, but the return to baseline is usually rapid. Viral infections, bacterial colonization, localized infections, allergic disorders, autoimmune diseases, and transplant rejection do not usually induce a significant PCT response (values < 0.5 ng/mL). Therefore, by evaluating PCT concentrations, the physician may use the findings to aid in the risk assessment for progression to severe sepsis and septic shock.

The results should be evaluated in context of all laboratory findings and the total clinical status of the patient. In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed.

13. PROCEDURE NOTES

- **FDA Status:** Approved
- **Validated Test Modifications:** None
  The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow up.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.05 – 200.00 ng/mL

14.2 Precision

Precision is assessed by analysis of plasma samples of known values, run in triplicate. The data appears consistent and all parameters have a low CV%.

14.3 Interfering Substances

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.

14.4 Clinical Sensitivity/Specificity/Predictive Values

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)	4g/dL
Human Calcitonin	60 ng/mL
Human Katacalcin	10ng/mL
Human a-CGRP	10µg/mL
Human b-CGRP	10µg/mL

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

PCT strip causes serious eye damage. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

16. RELATED DOCUMENTS

- Safety Data Sheets
- Biomerieux Vidas 3 Reference Manual
- Quality Control Program policy
- Laboratory Safety Manual
- VIDAS 3 Maintenance Log (AG.F394)
- Current package insert for VIDAS® B·R·A·H·M·S PCT

17. REFERENCES

1. VIDAS System package insert. Refer to the insert for the complete details of the procedure, references, and performance of this product.
2. Package insert, VIDAS® B·R·A·H·M·S PCT, BIOMERIEUX, 02/2017
3. Package insert, Lyphocheck Specialty Immunoassay Control, Bio-Rad Laboratories, 04/2019

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
0	7/19/19	14.1	Corrected upper AMR value	L Barrett	R SanLuis
1	8/14/20	Header	Changed WAH to WOMC	L Barrett	R SanLuis
1	8/14/20	6.3	Added steps to transmit QC	M Sabonis	R SanLuis
1	8/14/20	8.3	Added auto-verification of results & reference to add. B	M Sabonis	R SanLuis
1	8/14/20	10.5	Deleted manual resulting	L Barrett	R SanLuis
1	8/14/20	17	Add QC information	L Barrett	R SanLuis
1	8/14/20	19	Add addendum B	L Barrett	R SanLuis

19. ADDENDA

A. VIDAS 3 Maintenance
B. Resending Results to DI-Instrument Manager

Addendum A

VIDAS 3 Maintenance

DAILY: Check Temperatures

- Go to System
- Display Temps
- Review Temps Listed for SPR, Tray, Cooling unit, and the internal temp to make sure they are in the range on the maintenance log

MONTHLY: Clean SPR Block

- Select **System** from Navigation Toolbar
- Select **Maintenance**
- Select **SPR block cleaning** from the Maintenance Configuration List Option
- Select **[START]** in the Action Bar. The SPR blocks are moved to the home position
- Turn **OFF** the instrument by pressing the power switch on the right side. Wait until the instrument has completely turned off.
- Select **Validate Step** to confirm
- Open the section flap doors
- Flip the SPR block towards you and hold it in this position to easily access all surfaces of the block
- Clean all surfaces with a decontamination wipe
- When cleaning the rear surfaces of the SPR block, press each SPR liner to clean the bottom
- Using a Dacron swab moistened clean the interior
- Using a Dacron swab clean the interior of each SPR liner with a 10% bleach solution
- On the VIDAS screen validate the completion of the SPR block cleaning
- Close the section flap doors
- Select **Validate Step** to confirm
- Turn ON the instrument and wait for it to initialize
- Remove and reinsert the racks to resolve errors
- After initialization of the instrument select Validate step
- Select COMPLETED

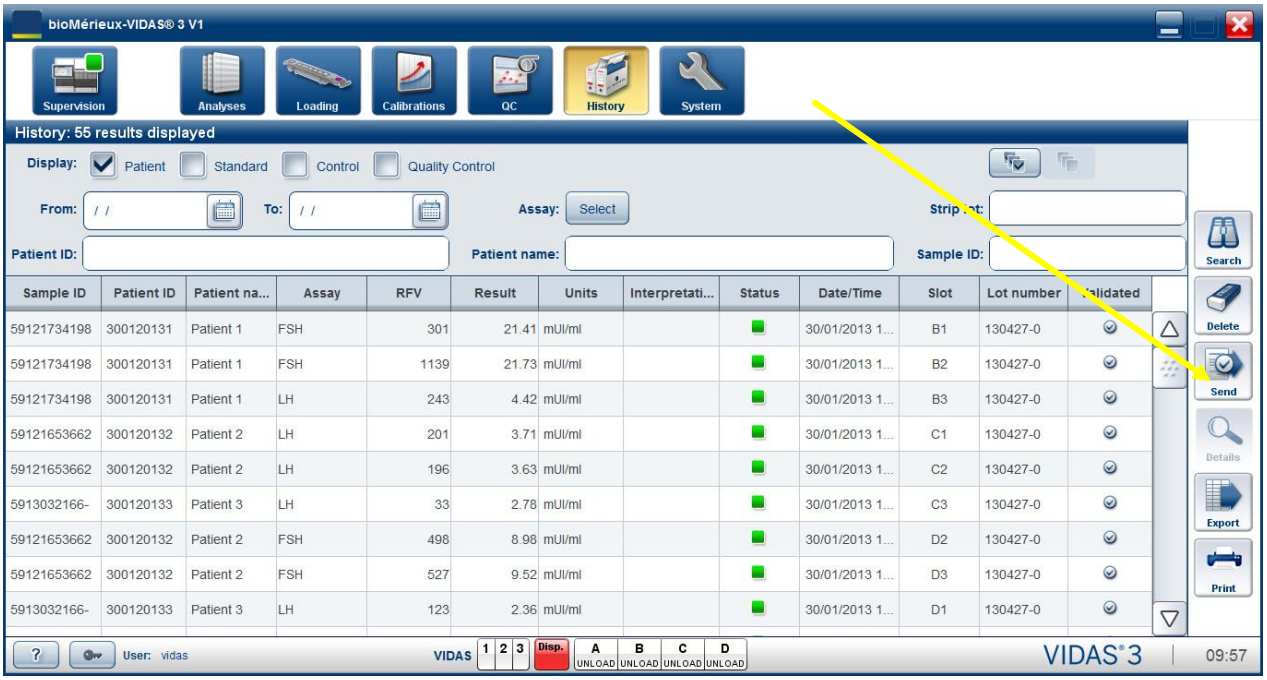
SIX MONTH MAINTENANCE: Clean Housing and Cover

- Clean vials, tubes and disposables
- Clean waste drawer
- Clean reagent strip sections
- Wipe off screen

Addendum B

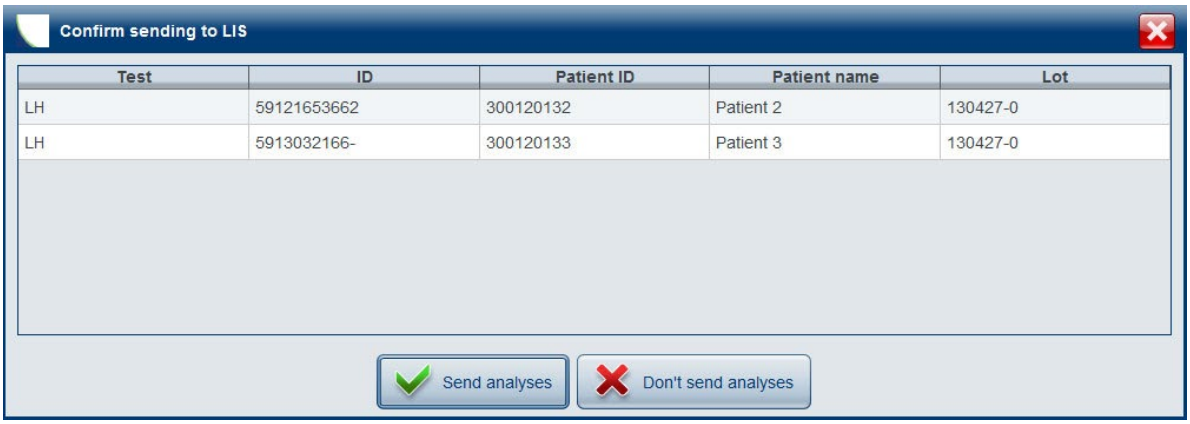
Resending Results to DI-Instrument Manager

1. Locate accession number via HISTORY.
2. Touch the accession and then touch the SEND button



Sample ID	Patient ID	Patient na...	Assay	RFV	Result	Units	Interpretati...	Status	Date/Time	Slot	Lot number	Validated
59121734198	300120131	Patient 1	FSH	301	21.41	mUI/ml			30/01/2013 1...	B1	130427-0	
59121734198	300120131	Patient 1	FSH	1139	21.73	mUI/ml			30/01/2013 1...	B2	130427-0	
59121734198	300120131	Patient 1	LH	243	4.42	mUI/ml			30/01/2013 1...	B3	130427-0	
59121653662	300120132	Patient 2	LH	201	3.71	mUI/ml			30/01/2013 1...	C1	130427-0	
59121653662	300120132	Patient 2	LH	196	3.63	mUI/ml			30/01/2013 1...	C2	130427-0	
5913032166-	300120133	Patient 3	LH	33	2.78	mUI/ml			30/01/2013 1...	C3	130427-0	
59121653662	300120132	Patient 2	FSH	498	8.98	mUI/ml			30/01/2013 1...	D2	130427-0	
59121653662	300120132	Patient 2	FSH	527	9.52	mUI/ml			30/01/2013 1...	D3	130427-0	
5913032166-	300120133	Patient 3	LH	123	2.36	mUI/ml			30/01/2013 1...	D1	130427-0	

3. A LIS dialog box displays for you to confirm that you want to send results to the LIS. Touch the "Send analyses" to send to LIS or Touch the "Don't send analyses" if you do not want to send to LIS. ** You MUST only send one result at a time.



Test	ID	Patient ID	Patient name	Lot
LH	59121653662	300120132	Patient 2	130427-0
LH	5913032166-	300120133	Patient 3	130427-0