



4095 Viral Hemorrhagic Fever - eg Ebola Sample Processing

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The VCH Intranet (<http://ipac.vch.ca/Pages/Ebola-Virus-Disease.aspx>) contains the most up to date information on VHF / EVD within VCH.

Intended use: The intended use of this document is to provide instructions specific to the Hematology Lab

General Guidelines:

Any laboratory staff involved in manipulating, pretreatment, processing, or testing of non- inactivated clinical specimens, including malaria smears, must do so in a class II biological safety cabinet (BSC) [**AFB Room 1103, Microbiology Lab**] with high level infection control precautions and PPE.

Refer to Laboratory Checklist for Donning/Doffing: ***E3. Donning and Doffing Checklist for VGH Laboratory Technologists.*** [OMNI VCH SAF Safety folder]

All patients will be screened by history and symptoms by ED triage staff. Identified suspect patients will be isolated in ED while ED MD screens further. Only moderate and high risk patients will be retained in isolation and handled with high risk protocol including lab measures as below.

ED will notify appropriate personnel including Medical Microbiologist on-call if a patient is considered Moderate/High Risk. Amongst others, Medical Microbiologist on-call will contact Hematopathologist on-call to notify hematology lab/ shift supervisor.

If approved, all lab handling of potentially virally active specimens must be done wearing full PPE, as outlined in checklist: ***E3. Donning and Doffing Checklist for VGH Laboratory Technologists.*** Any manipulation of the specimen, including the removal of the cap, must be done in the BSC type II.

Procedure – PRE examination

Refer to Microbiology SOP, "***E4. Ebola Processing Protocol***" - *VGH Laboratory Specimen Processing Guidelines: Patients with Suspect Ebola Virus Disease [EVD] or Viral Hemorrhagic Fever [VHF]*", for complete details.

Of note:

- Nursing staff will always collect blood samples from suspect EVD/VHF patients at VGH.** Samples will be labelled, disinfected and transported to laboratory in TDG (Transportation of Dangerous Goods) sealed hard plastic containers.
- Samples to be hand delivered by an MLA according to phlebotomy SOP – no use of pneumatic tube. Samples must never be left unattended.

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Documents used outside of OMNI are uncontrolled.

3. Lab receipt of samples will occur in designated area only: Microbiology AFB room with Biosafety Cabinet (BSC), class II.
4. Extra labels will be found in a separate bag at Microbiology Accessioning bench. Microbiology MLA will log in tests using the extra labels, NOT the specimens.
5. Only limited testing is permitted on patients until Ebola VD/VHF is excluded by PCR (BCCDC) and include:
 - i. Chemistry – [test code **VHFRMP**] -provided by the Piccolo analyzer (comprehensive metabolic panel): Na, K, TCO₂, Cl, Glucose, Ca, BUN, Creat, ALP, ALT, AST, TBIL, ALB and TP.
 - ii. Hematology -[test code **HEMRP**]
 - a. Malaria screen – thin films and rapid falciparum (MALR) test only
 - b. HB by Hemocue
 - c. WBC, PLT estimate
 - iii. Blood cultures

Procedure –examination

Upon lab notification, Microbiology will “prep” the AFB room and place the “EVD/VHF Supplies bin” containing many of the required supplies into the room. Full AFB Supplies list in document: “*AFB Room Supplies Lists – Suspect EVD/VHF Specimen Processing*”

Print updated SOPs from OMNI – no paper SOPs are maintained in AFB room.

- SOP 4095 Ebola VD/VHF Sample Processing - Hematology Lab
- SOP 4090 (Regional) Malaria Rapid Test – NCS Method
- SOP 4210 Hemocue Hb 201+
- SOP 2025 Piccolo Xpress Procedure

Read SOPs before proceeding to re-familiarize yourself with process.

The “buddy system” is mandatory for assistance in donning and doffing PPE, as well as sample processing.

Additionally, **bring the following with you to AFB Room:**

- One malaria rapid test
- An aliquot of malaria buffer in a capped tube (approx. 50 uL)
- 5 fine-tipped plastic transfer pipets from bone marrow room
- 2 Disposable Coplin jars with covers
- ~75 mL Methanol and ~75 mL 10% buffered-formalin (flammable cabinet BM room).
*Pour off into capped jars for safe transport.
- Forceps (plastic)
- Piccolo analyzer [*if known Ebola case previously processed, analyzer is found in Microbiology*]
- 2 Piccolo test disks [*box of test disks found in 2 door fridge by monospot kits*]
- Hemocue analyzer [*if known Ebola case previously processed, analyzer is found in Microbiology*]
- A bottle of Hemocue HB microcuvettes [*stored in Hemocue drawer, Diff bench*]
- A capped aliquot of a “normal” CBC EDTA blood – to act as a control for the Hemocue

- write the Hb result on the tube.
- Plastic slide transporters

Frosted slides are stocked in AFB room – on cart or shelf.

Microbiology Technologist:

Technologist must be trained in PPE use and have had a recent N95 fit check in the last year. They will prepare the BSC & AFB room for sample processing, and will assist Hematology technologist with donning and doffing the appropriate PPE. They will also provide support to the Hematology technologist during the testing process.

Technologist dons appropriate PPE in Microbiology Lab Main room donning area, just outside AFB room, assisted by Hematology Technologist, then assists Hematology technologist with donning of PPE. Enters AFB room and closes door.

Hematology Technologist:

Technologist must be trained in PPE use and have had a recent N95 fit check in the last year. Technologist dons appropriate PPE in Microbiology Lab Main room just outside AFB room, assisted by Microbiology Technologist, then assists Microbiology technologist with donning of PPE. Enters AFB room and closes door.

***PPE must include:**

- Extended cuff gloves (double gloved [use of two pairs])
- Fluid-impervious gowns
- Fluid-impervious shoe covers
- Full face shield
- N95 respirator – perform fit check.
- Fluid-Impervious head cover (hood)
- Bouffant Cap

*** Ensure only VCH approved PPE used – refer to final approved listing of EVD/VHF high level PPE – refer to VCH Approved listing on VCH IPAC website: <http://ipac.vch.ca/Pages/Emerging-Issues.aspx>**

Legend:

- Accel INTERVention disinfectant wipes = 'disinfectant wipes'
- Accel 5 TB disinfectant = 'disinfectant' (liquid)
- Alcohol abased hand rub = 'ABHR'

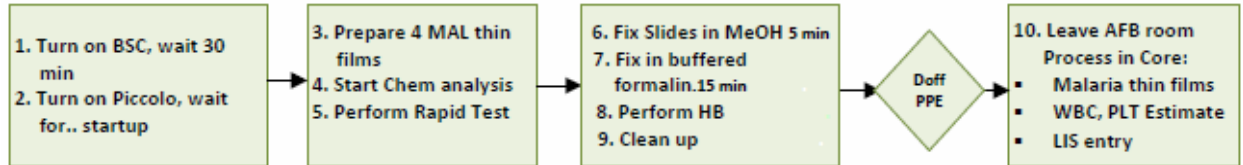
Contact Time:

- Accel INTERVention wipes require a **ONE minute** contact time
- Accel 5 TB disinfectant requires a **FIVE minute** contact time

Each time a specimen tube is removed from the TDG container, the exterior of both the TDG container and specimen tube are wiped with an Accel INTERvention disinfectant wipe.

Each time your hands move outside the BSC to perform another task, disinfect outer glove surface with disinfectant wipe or alcohol-based hand rub. Allow to dry.

To optimize time usage, the Hematology technologist will follow this testing process:



Buddy system mandatory – Hematology along with Microbiology technologist ‘buddy’ will enter the AFB room. The ‘buddy’ will read out from the checklist while the Hematology technologist performs the following:

→ **Proceed with testing:**

1. Chemistry Testing – Piccolo Express Point of Care Analyzer

All Chemistry testing will be performed on the Piccolo Express analyzer within the Biological Safety Cabinet, BSC II.

Testing is performed on Li Heparin whole blood (light green top tube).

Prior to entering the AFB room, ensure monthly QC has been run and within limits.

Note: if analyzer has previously processed a positive Ebola sample, all QC must be processed within the BSC while wearing the appropriate full PPE.

√	Buddy reads and checks after completion	
<input type="checkbox"/>	1	Turn on Piccolo analyzer to initialize. Piccolo is in ‘ready state’ when the [Analyze] option becomes available.
<input type="checkbox"/>	2	Carefully remove test disk from foil pouch. Do NOT touch the optical surfaces of the disk – hold the disk as you would a CD, by gently holding by its edges. Inspect for damage / scratches.

<input type="checkbox"/>	3	<p>In BSC, mix by inversion, then open the Li Heparin whole blood sample. Place cap on a disinfectant wipe.</p> <p>Using a fine tipped transfer pipette, dispense ~100 uL whole blood into the sample port of the test disk (~100 uL will fill the pipette to the 2nd hub).</p> <p>Fill until the sample reaches the sample fill line of the test disk – do not introduce air bubbles or air gaps into the disk.</p> <p>If sample overflows, gently wipe away excess with adequate lint-free cloth to avoid gloves coming into contact with blood.</p> <p>Recap tube, wipe with disinfectant wipe. Wipe gloves with a fresh disinfectant wipe and allow to dry.</p>
<input type="checkbox"/>	4	Press the [Analyze] option on the Piccolo touch screen to open the disk drawer.
<input type="checkbox"/>	5	Place disk in drawer and press the close button. Analysis of sample begins.
<input type="checkbox"/>	6	Select the specimen type on the touch screen: Patient or Control .
<input type="checkbox"/>	7	Enter the sample ID using touch screen keypad.
<p>Analysis will take approximately 12 minutes.</p> <p>— During this 12 minute window, other testing can proceed (Proceed to Section 2) —</p>		
<input type="checkbox"/>	8	When analysis is complete, results will automatically print (and be stored in Piccolo database).
<input type="checkbox"/>	9	<p>Press [Open] to remove the test disk.</p> <p>Discard into double-bagged biohazardous waste container in BSC.</p>
<input type="checkbox"/>	10	Wipe the disk drawer with a disinfectant wipe. Allow to dry. Repeat this step again.
<input type="checkbox"/>	11	Press [Close] to close the disk drawer.
<input type="checkbox"/>	12	<p><u>Result documentation:</u></p> <p>The ‘buddy’ will complete Appendix A - VPCO Piccolo Express Worksheet by documenting the results, <u>as read out by the performing technologist</u>, in the same order as the print out.</p> <p>The recorder must read back the results to the performing technologist.</p> <p>If IQC (Internal QC) is not within specifications, repeat test. Otherwise, no repeats are required.</p> <p>If an analyte generates an error and you are unsure how to proceed, refer to SOP Piccolo Express</p> <p>The VPCO Piccolo Express Worksheet will be given to the Vista Bench in Core lab Chemistry for LIS entry.</p>
<input type="checkbox"/>	13	<p>Turn off and unplug the Piccolo.</p> <p>Wipe exterior of analyzer including AC adaptor & power cord with disinfectant wipes. Allow to dry. Repeat this step again.</p>

<input type="checkbox"/>	14	<p>The Piccolo will be stored with the EVD/VHF supplies in the AFB room until BCCDC testing for Ebola VD/VHF is complete.</p> <p>If the BCCDC test is <u>negative</u>, the analyzer will be stored in the Core Lab.</p> <p>If the BCCDC test is <u>positive</u>, the analyzer will be stored in the AFB room, double-bagged in red biohazard bags, indefinitely. Label the outer bag in large lettering: “Contaminated with Ebola Virus Disease/Viral Hemorrhagic Fever. Do not remove from AFB room.”</p>
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2. Malaria Rapid Test & Thin Film preparation

Specimen is EDTA whole blood (lavender top tube) – testing will be by rapid test (dipstick for falciparum) and thin films. Technologist must continue wearing PPE.

√	Buddy reads and checks after completion	
<input type="checkbox"/>	1	In BSC, mix gently, and open EDTA sample - place the cap on top of a disinfectant wipe.
<input type="checkbox"/>	2	<p>Perform rapid dipstick test in BSC as per SOP (ask buddy for instructions as required). [Sample into sample well A + 2 drops buffer to window B; read at 20 min] (Buddy records time).</p> <p>Interpret result and dispose of dipstick and extra buffer in the double-bagged waste container within BSC.</p> <p>Record result on Appendix A result worksheet.</p>
<input type="checkbox"/>	3	<p>Using a fine-tipped plastic transfer pipet, make 4 thin spread films in BSC. Ensure they are of acceptable wedge length as they will be scanned on Cellavision for PLT estimate.</p> <p>Discard spreader into small sharps container & pipet into the double-bagged waste container within BSC. Recap tube, wipe with a disinfectant wipe. Wipe gloves with a fresh disinfectant wipe and allow to dry.</p>
<input type="checkbox"/>	4	<p>Place air-dried thin films in the disposable Coplin jar filled with methanol. Ensure that the entire slide length is immersed.</p> <p>Fix in methanol for 5 minutes (Buddy records time).</p>
<input type="checkbox"/>	5	<p>After 5 minutes, transfer slides, using forceps, to the disposable Coplin jar filled with 10% buffered formalin. Ensure the entire slide length is immersed.</p> <p>Fix in formalin for 15 minutes. (Buddy records time).</p>
<input type="checkbox"/>	6	After fixation, disinfect outer glove surface with a disinfectant wipe and allow them to dry.
<input type="checkbox"/>	7	Using forceps, remove slides from 10% buffered formalin and lean them up against sample rack to air dry for ~ 5 minutes. Once dry, place in cardboard / plastic slide holders with an “inactivated” sticker affixed to the slide holders.

<input type="checkbox"/>	8	<p>Once testing is complete, wipe down outside of Coplin jars with disinfectant wipes, allow to dry, and set Coplin jars aside in the BSC. Coplin jars will remain in the BSC until BCCDC testing for Ebola VD/VHF is complete.</p> <p>If the BCCDC test is <u>negative</u>, pour contents of jars down dirty sink with running water in AFB room and place jars in wash-up bin in bone marrow room.</p> <p>If the BCCDC test is <u>positive</u>, place capped Coplin jars with contents in double-bagged biohazardous waste in BSC.</p>
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3. HB by Hemocue

Refer to SOP 4210 Hemocue 201+ as required.

If analyzer is stored in Core Lab (has not yet processed a positive Ebola VD/VHF sample), QC may be run in Hematology before entering AFB room.

Note: if analyzer has previously processed a positive Ebola VD/VHF sample, all QC must be processed within the BSC Class II while wearing the appropriate PPE.

√	Buddy reads and checks after completion	
<input type="checkbox"/>	1	<p>In the BSC, place the Hemocue Hb 201+ analyzer and plug in the power adaptor.</p> <ul style="list-style-type: none"> ▪ Pull the cuvette holder out to the loading position. ▪ Press and hold the On / Off button (left button) until the display is activated. ▪ The display shows the version number, and then displays an hourglass and “Hb”. ▪ After ~ 10 s, three flashing dashes appear with HemoCue symbol → ready to use.
<input type="checkbox"/>	2	<p>Run the QC sample – using the ‘normal’ EDTA aliquot on which the HB result has been written.</p> <ul style="list-style-type: none"> ▪ Acceptable QC: $\pm 7\%$ of Sysmex value. ▪ Record result on Appendix A, result worksheet. ▪ After analysis, discard QC aliquot in double-bagged biohazardous waste in BSC.
<input type="checkbox"/>	3	<p>Analyze patient:</p> <ul style="list-style-type: none"> ▪ Mix an EDTA sample from the sample rack by inversion minimum 8 times. ▪ Remove stopper and place on a disinfectant wipe. Using a fine-tipped plastic pipet, remove ~ 25 uL of blood and place it on a microscope slide. Discard fine-tipped pipet. Recap tube, wipe with a disinfectant wipe. Wipe gloves with a disinfectant wipe and allow to dry. ▪ Open the bottle of microcuvettes and remove one. Close lid promptly. (The buddy may aid with this step).

		<ul style="list-style-type: none"> ▪ Place microcuvette near drop of blood on slide and allow cuvette to fill by capillary action. ▪ Using 2x2" gauze or Kimwipes, wipe the exterior of the microcuvette taking care not to touch the open end of microcuvette. <ul style="list-style-type: none"> ○ There should be no air bubbles in the optical eye of cuvette. ▪ Place the filled cuvette into the cuvette holder and gently slide the holder into the measuring position. ▪ During measurement, an hourglass will be shown on the display. <ul style="list-style-type: none"> ○ The result will be displayed within 15 - 60s and will remain on the display as long as the cuvette holder is in the measuring position. ▪ Pull the cuvette holder out to the loading position. Remove the microcuvette and discard it in the biohazard container within BSC. Wipe gloves with a fresh disinfectant wipe and allow to dry. ▪ Record result on Appendix A, result worksheet.
<input type="checkbox"/>	4	<p>Unplug the Hemocue. Using disinfectant wipes, wipe down and allow to air dry the following components two times:</p> <ul style="list-style-type: none"> ▪ the microcuvette loading bay ▪ the exterior of Hemocue ▪ the adapter wire cord ▪ the exterior and cap of Hemocue microcuvette bottle
<input type="checkbox"/>	5	<p>The Hemocue will be stored with the EVD/VHF supplies in the AFB room until BCCDC testing for Ebola VD/VHF is complete.</p> <ul style="list-style-type: none"> ▪ If the BCCDC test is negative, the analyzer will be stored in the Core Lab. ▪ If the BCCDC test is positive, the analyzer will be stored in the AFB room, double- bagged in red biohazard bags, indefinitely. Label the outer bag in large lettering: "Contaminated with Ebola Virus Disease/Viral Hemorrhagic Fever. Do not remove from AFB room. "
<input type="checkbox"/>	6	<p>The Hemocue bottle of microcuvettes should STAY in the BSC until BCCDC testing is complete.</p> <ul style="list-style-type: none"> ▪ If the BCCDC test is negative, it will be stored in the Core Lab. ▪ If the BCCDC test is positive, it will be stored in the AFB room, double- bagged in red biohazard bags until expiry date or simply discarded. Label the outer bag in large lettering: "Contaminated with Ebola Virus Disease/Viral Hemorrhagic Fever. Do not remove from AFB room. "

4. Exiting AFB room to Core Lab

- i. Very carefully, with assistance from Microbiology Technologist, doff PPE prior to exiting AFB room according to checklist: 'E3. Donning and Doffing Checklist for VGH Laboratory Technologists'.
- ii. Transport to the Core Lab:
 - Malaria slides using cardboard / plastic slide holders with an "inactivated" sticker affixed to the slide holders.
 - Tests are accessioned / received by Micro MLA. If not, seek assistance

from Lab Reception as required for proper admission, account use.

Malaria thin films

- i. Swirl the slides gently in a beaker of Sysmex buffer, pH 7.15 (found in BM room) for ~30 seconds and allow to dry.
- ii. Stain with Wright-Giemsa on Hematek stainer. Follow malaria SOP from this point forward. Do not make dilute giemsa-stained slides or thick films.
Note: do not wipe front side of slide other than very gently – do not remove oil, films have a tendency to rub off.
- iii. Contact Hematopathologist on call to read and comment.
 - Thin films fixed with formalin are considered inactivated and safe to transport back to hematology lab for interpretation.
 - Dipstick need not be repeated after initial testing.
 - Thin films negative for malaria may be repeated q8-12h x3 to completely exclude.

WBC Estimate – 2 technologists

- i. Using a stained malaria slide, perform a WBC estimate by scanning approximately 10 fields on low (**10x**) power.
- ii. Average the number of WBC and multiply by **0.2** to obtain WBC estimate.
(if using 40x, multiply by 2)
 - if WBC > 3.0, the estimates should check within $\pm 30\%$
 - If WBC < 3.0, the estimates should check within $\pm 50\%$
 - If they don't check, a 3rd technologist should perform an estimate.
↳ See Reporting Section

PLT Estimate

- i. Print a mini slide barcode label. Label a stained malaria slide(s) and load and scan on CellaVision.
- ii. Using the PLT tab of 16 grids, count the number of PLT in each square and input that value into the fields.
- iii. Press [Calculate PLT estimate] to obtain PLT estimate.
 - If grids are highly variable, and count is questionable, perform estimate on a 2nd slide.
↳ See Reporting Section

RBC Morphology

- i. Using a stained malaria slide, an RBC morphology may be performed if on scanning, anything of reportable significance is seen.

↳ See Reporting Section

5. Clean Up / Decontamination of BSC in AFB room

- i. Hematology will:
 - Ensure all consumables, disposables, gauze, towels have been placed in the double- bagged waste container in BSC.
 - Ensure Hemocue and Piccolo Express analyzer have been disinfected as outlined in Section 3, Step (4), placed inside red biohazard bags (double bagged): tie off bags with twist ties - one inside the other, and put in Ebola VD/VHF Supplies box for storage in AFB room
 - Assist Microbiology technologist as needed.
- ii. Microbiology will:
 - Ensure proper decontamination of interior of BSC
 - Autoclave all waste
 - Ensure Ebola VD/VHF Supplies box is re-stocked with inventory.

Procedure – Reporting Results

- i. In the Core lab, using Appendix A - Result Form, manually enter:

Worksheet	Test code	notes
MAL	MALTF	Thin film
	MALR	Rapid test
	MALC	Leave blank for Hematopathologist
CBC	WBC	Append ESTIM (Count obtained by estimation on stained blood film)
	HB	
	PLA	Append ESTIM (Count obtained by estimation on stained blood film)
	if CBC ordered instead of individual tests, enter RBC, HCT, MCV, MCH, MCHC, RDW, MPV, CBCMT as HIDE	

- ii. RBC morphology – if required, use Sunquest GUI keyboard or add-on and report a MORFX.
- iii. Chemistry – Results transcribed to “VPCO Piccolo Express Worksheet” will be handed to a Chemistry technologist for interpretation / reporting.

Procedure – POST examination

Once EVD/VHF is excluded by BCCDC:

- Laboratory will be notified and further samples from this patient can be treated with routine universal precautions. Additional testing (beyond that listed above) will then be allowed.
- Dispose into the double-bagged waste container located within the BSC the following:
 - All samples after testing is complete
 - All disposables (pipettes, tubes, caps, etc)
 - All printed SOPs
 - Everything brought inside the BSC during processing (except centrifuge accessories)
- Leave TDG containers in Micro AFB area
- Return Hemocue, bottle of microcuvettes, Piccolo Xpress analyzer to Core Laboratory

Appendix A – VPCO Piccolo Express Worksheet and Test Information

Affix barcode label:		HB (HCUE) result: _____				Performed by:	
		MALR rapid test: _____				Recorded by:	
		HB QC: XN _____ HCUE _____					
Test	Enter Piccolo printout result	File as		* Verify limit Phone if		Technical limit	Filed by: / date / time
		<	>	<	>		
NA mmol/L		110	170	120*	160*	110 - 170	
K mmol/L		1.5	8.5	2.8*	6.2*	1.5 – 8.5	
TCO2 mmol/L		5	40	10*	40*	5 - 40	
Cl mmol/L		80	135	–	–	80 - 135	
GLUC mmol/L		0.6	38.9	2.0*	30.0*	0.6 - 38.9	
Ca mmol/L		1.00	4.00	1.50*	3.25*	1.00 – 4.00	
URE (BUN) mmol/L		0.7	64.3	–	–	0.7 – 64.3	
CRE umol/L		18	1768	–	>500*	18 - 1768	
EGFR		–	–	–	–	–	
ALKP (ALP) U/L		5	2400	–	–	5 - 2400	
ALT U/L		5	2000	–	–	5 - 2000	
AST U/L		5	2000	–	–	5 - 2000	
TBIL umol/L		2	513	–	–	2 - 513	
ALB g/L		10	65	–	–	10 - 65	
TP g/L		20	140	–	–	20 - 140	
AGAP	LIS Calculated	<1.0	–	2.0	26.0		
VHFDIS	Auto Comment:	WBPOC = Analysis was performed on a whole blood specimen using a point of care device.					
VHFCMT	Auto HIDE	VHFCMT = test interpretation - do not result					