Interim Laboratory Testing Guidance: BioFire Global Fever and Warrior-CA Panels for Screening of Select High Consequence Pathogens

The recommendations contained in this document should be read in conjunction with relevant federal, provincial, territorial and local legislation, regulations, and policies. Recommended measures should not be regarded as rigid standards, but principles and recommendations to inform the development of guidance and harmonize the testing process between provincial laboratories.

This guidance is based on consensus agreement within the Canadian Laboratory Response Network (CLRN) BioFire Working Group, select subject matter experts and currently available scientific evidence, adopting a precautionary approach where the evidence is lacking or inconclusive. It is subject to review and change as new information becomes available.

Any testing performed by the CLRN laboratories outside of the recommended parameters outlined in this document is done at the discretion of the laboratory performing the testing, recognizing that there may be limitations and caveats.

Scope:

This document provides guidance on the laboratory testing criteria and specimens to be collected for symptomatic persons with potential exposure to high consequence pathogens (HCP), using the BioFire® FilmArray® system and the BioFire Global Fever (GF) Panel (Research Use Only, RUO) and/or Warrior-CA Panel (in vitro Diagnostic use, IVD).

Evaluation was completed for the following Viral Hemorrhagic Fever (VHF) targets:

- Crimean-Congo hemorrhagic fever virus
- Ebolavirus *(Bundibugyo, Reston, Sudan, Tai Forest, Zaire)**
- Lassa virus
- Marburg virus*

Evaluations were completed for other priority targets of interest:

- Chikungunya virus
- Dengue virus
- West Nile virus
- Yellow Fever virus
- Zika virus
- *Plasmodium* spp.*, including Plasmodium falciparum and Plasmodium vivax/ovale*

***Warrior-CA panel includes only Ebolavirus and Marburg targets from this HCP list**

Background:

The Canadian Laboratory Response Network within the National Microbiology Laboratory Branch (NML) distributes quality controlled, high confidence assays for the detection of security sensitive biological pathogens to Canadian provincial stakeholders in support of front-line preparedness and response efforts. Sporadic outbreaks of filoviruses have been occurring on an almost annual basis, including three that occurred in 2025 (Sudan virus in Uganda, Ebola virus in DRC, and Marburg virus in Ethiopia), and one in 2026 (Bundibugyo virus in DRC and Uganda). Furthermore, thousands of cases of Lassa virus and Crimean-Congo Hemorrhagic Fever virus occur each year, adding to the risk of potential travel-related cases in Canada. To increase screening capabilities at the provincial level, and leveraging the deployment of BioFire® FilmArray® instruments in Canada during the COVID-19 pandemic, a multicentre verification study (MCVS) of BioFire test panels was undertaken to support the triage of High Consequence Pathogens (HCPs) that are circulating outside of Canada but pose potential risk of import. While the NML maintains specialized reference and diagnostic services for these HCPs vis-a-vis high containment, distribution of well-characterized, robust assays to be utilized at provincial node laboratories to screen high-risk patients greatly increases Canada's ability to detect and treat in a timely manner, in the interest of the patient, public health and public safety. These multiplexed assay test pouches further expand diagnostic capabilities by enabling rapid detection of other rare infectious agents within Canada, including *Plasmodium* spp. (the causative agent of malaria), and facilitating a timely diagnosis, which is an important consideration for travelers returning to Canada.

Understanding the performance criteria of these tests, and limitations, informs best-use guidelines of this technology for the rapid triage for high consequence diagnostic specimens across Canada. To date, ranges for the BioFire® GF Panel's limit of detection for each target have only been reported as conference proceedings and not in peer reviewed publication (5-8). There are limited published data describing clinical evaluation of the specificity and sensitivity of individual targets of the GF panel (9-12). As such, the MCVS was conducted by the CLRN to verify performance and the summary of the limit of detection for prioritized targets can be seen in Appendix 1.

The CLRN and its partners are working to support the laboratory response through the production of these interim recommendations. This guidance document will focus on 1) defining testing criteria for individuals at risk of HCPs and 2) recommendations related to the sample collection and testing for detection of a HCP.

Patient Testing Criteria:

Patients must have a compatible acute illness with features suggestive of HCP, together with epidemiologic risk factors for exposure (e.g. recent travel to an endemic area, area with an established or evolving HCP outbreak, neighboring country or documented reservoir contact, or close contact with a person with suspected or confirmed HCP).

If a VHF is suspected, best management practices must be followed. Laboratories should refer to the WHO guidelines and supplement with their existing provincial guidance, as necessary. Clinical features, laboratory diagnosis, and management for specific VHFs – Ebola, Marburg, CCHF, and Lassa fever – are presented in the WHO pocket guide (1, 2) and summarized here:

> Clinical criteria: Any of acute fever, headache, myalgia, weakness/fatigue, sore throat, loss of appetite, vomiting, diarrhea, abdominal pain, rash, or any unexplained bleeding/bruising, petechiae/ecchymoses, hematemesis, melena, bloody diarrhea, jaundice, shock, altered mental status, or rapid progression to multiorgan failure.
>
> Epidemiologic criteria: Includes but not limited to travel to a VHF-affected area, especially with active transmission; direct contact with a sick or dead person(s); contact with blood/body fluids of person(s) in affected area; exposure to contaminated bedding, medical equipment, or animal reservoirs; or known laboratory/healthcare exposure.

The use of BioFire testing is intended as an adjunct triage tool within existing provincial viral testing algorithms and does not replace NML reference testing to meet international health regulations (IHR).

Sample Collection and Testing

Diagnostic testing for HCPs using the BioFire assays at the provincial reference laboratories should be performed only after consultation with appropriate public health officials and completion of a risk assessment. When any Risk Group 4 (RG4) VHF virus is suspected, the NML Special Pathogens (SP) Reference Program should be informed. For other priority targets of interest, the decision to contact the Vectorborne Zoonotic and Diseases (VBZD) Reference Program rests with the province, following their standard testing procedures. Each laboratory with BioFire capability for HCPs should work through an internal risk assessment for local use, inclusive of specimen handling, testing and reporting (footnote). Reference testing by the NML Reference Program is required to meet IHR requirements where a VHF remains a concern. A negative BioFire result can contribute to local de-escalation when the combined clinical, epidemiologic, and laboratory assessments

supports low risk, however, concurrent testing at the NMBL is recommended for any suspected negative specimens. This aligns with best practices where negative results must be interpreted in context and not in isolation. Ongoing risk assessment based on the evolving clinical presentation and exposure/epidemiologic criteria remains essential.

The BioFire® GF Panel and Warrior-CA Panel are closed-systems with a disposable pouch that stores all the necessary reagents for nucleic acid extraction, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple pathogens within a single whole blood specimen (3, 4). Clinical specimens from patients under investigation for these HCPs may be tested using the BioFire® panels within a CL2 laboratory using a certified biological safety cabinet and enhanced biosafety precautions, following PHAC's laboratory biosafety guidelines and advisory on handling specimens from suspected HCP cases.

Specimen and testing recommendations for BioFire assays

- Blood: minimum sample volume of 200uL of whole blood is required per test. Refer to appropriate instructions for use document for specific requirements (3, 4).
    - Collection of 1mL of sample is ideal in case of requirement for repeat testing or referral.
    - Heparin is not recommended for the GF panel as it may interfere with results.
    - Repeat testing should be considered when initial nucleic acid amplification test (NAAT) results are negative but clinical suspicion remains high.
    - A second sample should be collected at least 24–72 hours after the first to reduce risk of false negatives in early disease. There is evidence that patients can test negative for RG4 VHFs up to 72hr, without a positive laboratory result until presenting obvious illness (1). The decision for obtaining a second sample should be based on known natural history of viremia/burden and test performance of the assay.
    - Samples can be held at room temperature for 1 day, or refrigerated up to 7 days (1).
- A 'not detected' result does not preclude the presence of a HCP, including VHF causing viruses not on the BioFire panels. Results must be interpreted in the context of the clinical and epidemiological information available. Concurrent testing at the NML is recommended.

- Confirmation for 'detected' and indeterminate results for VHFs on the BioFire® panels must be performed at the NML, based on nucleic acid amplification testing, used alone or in combination with sequencing.

- Testing guidance for the NML can be found here:
  Special Pathogens - Guide to Services - CNPHI
  Vector-borne and Zoonotic Diseases - Guide to Services - CNPHI

Limitations of testing

The GF panel target limits of detection were verified for prioritized targets via a Canadian MCVS in collaboration with the NML (Appendix 1). The differences seen between the observed limit of detection values in the MCVS and the manufacturer reported ranges may be attributed to several factors including the specific strains tested and differences in sample preparation and handling. Limitations of a nucleic acid assay must be considered within context. False negative results can arise from multiple mechanisms, including technical error, genetic variation in the target region, the presence of assay specific PCR inhibitors, suboptimal specimen collection (e.g. inappropriate timing or insufficient volume), and other pre-analytical or analytical factors. Conversely, false positives may also occur.

The MCVS was conducted using contrived samples, however there have been limited clinical evaluation studies comparing the GF panel to other molecular diagnostic methods that should be considered as well. A large multicentre prospective study (1,875 patients worldwide) reported high diagnostic accuracy of the GF panel for six analytes - chikungunya virus, dengue virus (serotypes 1–4), *Leptospira spp.*, *Plasmodium spp.*, *P. falciparum*, and *P. ovale/vivax* (9). Sensitivity ranged from 92.7-100% and specificity was above 99.2% for all targets against comparative diagnostic PCRs. Both false positive and false negative specimens were identified; in 56 of 72 cases, additional testing indicating these results were associated with pathogen concentrations near the limit of detection of the GF assays. Similar to other studies, diagnostic limitations were linked to short detection windows in blood and delays in clinical presentation.

Another study compared the GF panel detection accuracy to conventional PCR methods for 10 analytes in 82 patients and found an overall specificity of 96.0% but positive agreement of only 85.71%, with reduced sensitivity observed for *Plasmodium falciparum/P. malariae*, leptospirosis, and *Salmonella Typhi/Paratyphi* (10). Delayed testing (3–15 days after symptom onset) was identified as a key factor contributing to reduced sensitivity and highlighted the limitation of relying on a single diagnostic approach. Conversely, a study

comparing the GF panel to Malaria Multiplex Sample Ready (MMSR) assay for *Plasmodium* spp. detection found that the GF panel was more sensitive (11).

Finally, a dengue surveillance study (224 patients) demonstrated that the GF panel performs comparably to qRT-PCR in the early phase of illness (days 1–4) with sensitivity above 94%, but specificity declined rapidly with days after infection (12). Discrepancies between methods, including both false positives and false negatives, were attributed to factors such as sample matrix variation, and underscored the need for a two test algorithm of testing samples negative by RT-PCR sequentially by GF.

Overall, studies to date consistently show that while the GF panel offers broad pathogen coverage and high specificity, its sensitivity can vary and the performance is influenced by timing of sample collection, clinical and technical factors. The evidence supports the use of the GF panel as part of a multi-method diagnostic strategy to improve detection and reduce the risk of missed infections. While false negative and false positive results are expected to be rare using the BioFire assays, all suspected VHF samples should be routed in real time to the NML for testing, regardless of result. Once NML receives the suspected VHF specimen, additional reference test results for the RG4 VHFs will be provided within the published turnaround time, typically 6-8 hours within receipt. Alternatively, NML maintains a mobile laboratory capacity, if required.

Footnote. Research Use Only Global Fever Panels are marked 'not for diagnostic use'. The panels can be used as a tool to triage and inform testing needs. The Warrior-CA panels are Health Canada approved for diagnostic use.

References

1. Clinical management of patients with viral haemorrhagic fever: a pocket guide for front -line health workers: interim emergency guidance for country adaptation. World Health Organization. ISBN 978 92 4 154960 8(NLM classification: WC 534).

2. Ebola disease: For health professionals, humanitarian aid workers - Canada.ca

3. FilmArray® Global Fever Panel – RUO Instructions for Use.

4. FilmArray® NGDS Warrior Panel – CA Instructions for Use.

5. Helm, Jared. R. et al. (2017, November). Analytical Performance of the FilmArray® Global Fever Panel [Poster presentation]. American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting, Baltimore, MD.

6. Helm, Jared. R. et al. (2018, November). Analytical Performance of the FilmArray® Global Fever Panel [Poster presentation]. American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting, New Orleans, LA.

7. Rabinger, David. S. et al. (2022, November). Clinical Evaluation of the BioFire® FilmArray® Global Fever Special Pathogens Panel [Poster Presentation]. American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting, Seattle, WA.

8. BioFire Defense, LLC. (2023). 510(k) Summary: BioFire Global Fever Special Pathogens Panel 9510K No. K213362.) U.S. Food and Drug Administration.

9. Manabe, Yukari. C. et al. Clinical evaluation of the BioFire Global Fever Panel for identification of malaria, leptospirosis, chikungunya, and dengue from whole blood: a prospective, multicentre, cross-sectional diagnostic accuracy study. Lancet Infect Dis. 2022 September ; 22(9): 1356–1364. doi:10.1016/S1473-3099(22)00290-0.

10. Borgans F, et al. Comparative evaluation of a multiplex polymerase chain reaction panel for high-consequence infectious diseases against conventional diagnostics in travelers with febrile diseases. Int J Infect Dis. 2025 Dec;161:108147. doi: 10.1016/j.ijid.2025.108147. Epub 2025 Oct 17. PMID: 41110593.

11. Leski TA, et al. Comparison of capillary and venous blood for malaria detection using two PCR-based assays in febrile patients in Sierra Leone. Malar J. 2021 Mar

6;20(1):133. doi: 10.1186/s12936-021-03644-y. PMID: 33676502; PMCID: PMC7936501.

12. Felices V, et al. Comparative Analysis of Molecular Detection Algorithms for Enhanced Dengue Surveillance: A Field Case Evaluation. Am J Trop Med Hyg. 2025 Dec 30;114(3):540-547. doi: 10.4269/ajtmh.25-0395. PMID: 41666434; PMCID: PMC12964850.

13. Shokoples SE, Ndao M, Kowalewska-Grochowska K, Yanow SK. Multiplexed real-time PCR assay for discrimination of Plasmodium species with improved sensitivity for mixed infections. J Clin Microbiol. 2009 Apr;47(4):975-80. doi: 10.1128/JCM.01858-08. Epub 2009 Feb 25. PMID: 19244467; PMCID: PMC2668309.

Appendix 1. Limit of Detection Comparison of the BioFire® Global Fever (GF) Panel Targets

Global Fever Target	BioFire Reported GF LoD Range (copies/mL)	CLRN Verified GF LoD (copies/mL)	RT-PCR assay LoD (copies/mL)	NML section
Chikungunya	5.5E+01 to 5.5E+02	1.0E+04	Target 1 = 1.0E+04 Target 2 = 1.0E+04	VBZD
CCHF	6.4E-01 to 6.4E+02	1.0E+03	Target 1 = 1.0E+03 Target 2 = 1.0E+03	SP
Ebola - Bundibugyo	7.0E+02 to 7.0E+04	1.0E+04	Target 1 = 1.0E+02 Target 2 = 1.0E+03	SP
Ebola - Reston	2.7E+03 to 2.7+E04	1.0E+05	Target 1 = 1.0E+05 Target 2 = 1.0E+04	SP
Ebola - Sudan	8.3E+01 to 7.0E+04	1.0E+02	Target 1 = 1.0E+03 Target 2 = 1.0E+03	SP
Ebola - Tai Forest	8.3E+01 to 8.3+E03	1.0E+04	Target 1 = 1.0E+05 Target 2 = 1.0E+05	SP
Ebola - Zaire	8.3E+01 to 7.0E+04	1.0E+02	Target 1 = 1.0E+02 Target 2 = 1.0E+03	SP
Dengue (Serovar2)	2.7E+01 to 2.7E+03	1.0E+01	Target 1 = 1.0E+02	VBZD
Lassa	1.1E+03 to 5.6E+04	1.0E+04	Target 1 = 1.0E+04 Target 2 = 1.0E+04	SP
Marburg	2.6E+01 to 5.0E+02	1.0E+02	Target 1 = 1.0E+01 Target 2 = 1.0E+01	SP
West Nile	1.6E+02 to 2.3E+04	1.0E+02	Target 1 = 1.0E+03 Target 2 = 1.0E+03	VBZD
Yellow Fever	1.2E+01 to 1.2E+02	1.0E+03	Target 1 = 1.0E+04 Target 2 = 1.0E+04	VBZD
Zika	1.3E+01 to 1.3E+02	1.0E+02	Target 1 = 1.0E+03 Target 2 = 1.0E+03	VBZD
Plasmodium spp	1.0E+01 to 2.4E+02	1.0E+03	Target 1 = 1.0E+04^	PHO*
P. falciparum	1.0E+02 to 1.0E+03	1.0E+03	Target 1 = 1.0E+04	PHO*
P. vivax/ovale	7.7E+01 to 2.4E+02	1.0E+02	Target 1 vivax = 1.0E+04 Target 1 ovale = 1.0E+04	PHO*

The BioFire reported Limit of Detection (LoD) range listed is a summary of results from multiple poster presentations (References 5-7) and the Global Fever Special Pathogens FDA 510k document (Reference 8). For the pathogens present in both panels, the targets of the Global Fever Special Pathogens Panel are the same as those included in the Global Fever RUO Panel.

BioFire and RT-PCR testing were performed on a 10-fold dilution series of control materials spanning the reported LoD. CLRN verified BioFire LoD is defined as the lowest concentration at which the target pathogen was consistently detected (100%) across the sites participating in the multicentre verification study. Depending on the pathogen, three or four individual sites were involved in replicate testing. The number of replicates tested across all sites at each dilution ranged from 1-16, with most replicates tested at the dilutions surrounding the determined LoD.

Lab developed RT-PCR testing was performed by the reference NML sections using the same control materials as the BioFire testing. The LoD is defined as the lowest concentration at which 100% of target replicates were detected (n=6).
*NML does not have a reference section for *Plasmodium* testing and so Public Health Ontario (PHO) performed comparative testing with an in house RT-PCR (13), n=9.
^Plasmodium spp target n=18 and only 17/18 replicates were detected but no higher concentration was tested.

Quantified irradiated strains used as control material in the study were: EBOV Bundibugyo – Uganda 2007, EBOV Sudan-Boniface, EBOV Zaire-C05, EBOV-Reston, EBOV-Tai Forest, Marburg-Angola, Lassa-Josiah, CCHF-IBR10200, CHIKV-wild type, Yellow Fever-17D, West Nile-New York, Zika-Puerto Rico.

Quantified genomic DNA used as control material in the study were: *Plasmodium falcipa*rum *and Plasmodium vivax/ovale*. Material was provided by the National Reference Centre for Parasitology, by Dr. Momar Ndao.