Inactivating Zaire Ebolavirus in Whole Blood Thin Smears used for Malaria Diagnosis

Todd Cutts¹, Bradley Cook¹, Guillaume Poliquin³,4, James Strong⁴,⁵, Steven Theriault¹,²,⁶*

1) Applied Biosafety Research Program, Canadian Science Centre for Human and Animal Health and J. C. Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, MB R3E 3P6, Canada and 745 Logan Street, Winnipeg, MB R3E 3L5, Canada
2) Department of Microbiology, The University of Manitoba, Winnipeg, MB R3T 2N2, Canada
3) Office of the Scientific Director General, Canadian Science Centre for Human and Animal Health, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, MB, R3E 3P6
4) Department of Pediatrics and Child Health, The University of Manitoba, Winnipeg, MB R3T 2N2, Canada
5) Diagnostics Unit, Special Pathogens Program, Canadian Science Centre for Human and Animal Health, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, MB R3E 3P6, Canada
6) Chief Applied Biosafety Research Program, Canadian Science Centre for Human and Animal Health and J. C. Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, MB R3E 3P6, Canada

Abstract

Malaria is an important mimic or co-infection in potential Ebola virus disease patients. We evaluated the efficacy of 100% methanol inactivating Zaire ebolavirus Makona variant for...
malaria thin smear preparation. We determined that 100% methanol completely inactivated
the virus after 15 minutes.

Main Body

The 2014-2015 West African Ebola outbreak has uncovered knowledge gaps in several areas
regarding the safe handling of high containment viruses in clinical environments, such as front-
line diagnostic laboratories. A specific area of concern is the safe preparation of blood smears
for malaria diagnosis. Symptoms of malaria such as fever, headache, nausea, vomiting diarrhea,
closely mimic those of early ebola virus disease (EVD) (1,2,3). Additionally, since both circulate
in the same geographic area, co-infection is a concern (2). Malaria can be rapidly fatal if left
untreated, and as such, empirical antimalarial treatments are recommended in hospitals and
Ebola Management Centers in affected areas of Africa (3). In other areas of the world, anti-
malarial agents for severe malaria (e.g. artemisin-based compounds) can be difficult to access
due to limited supply or geographic distance (4). It is therefore important for laboratories to be
able to provide malaria smears safely concomitant with Ebola diagnostic results.

The Giemsa and Wright (Giff-Quik) staining techniques for malaria parasites in blood
samples are robust diagnostic methods easily performed in most laboratory settings. Prior to
staining, blood samples are prepared in either a thin and/or thick smear manner. Thick smears
can better detect the presence of the plasmodium than thin smears but are not methanol fixed,
while the thin smears can be fixed in methanol and provide the benefit of detection and
malarial speciation (6,7,12,13). However, it has not been definitively demonstrated that a
methanol fixation step prior to a Giemsa or Wright staining procedure is sufficient to inactivate

*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca, 1015 Arlington St Winnipeg Manitoba R3E
3R2 204-784-7505
Zaire ebolavirus (EBOV) in suspect malaria samples. Since both agents circulate within the same areas, have similar symptoms, and can co-infect, diagnostic samples require safe handling to prevent potential EBOV infection in health care workers. In this study, we evaluate the effects of methanol and a methanol-heat treatment against the EBOV/Mak variant using thin smeared slides containing human whole blood spiked with EBOV/Mak in the absence of the Plasmodium parasites.

Virus stock concentrates were prepared as previously described (5). The whole blood from a volunteer was drawn and stored at 4°C. An aliquot of whole blood was spiked with concentrated EBOV/Mak virus preparation (5) to attain a final concentration of $10^8$ TCID50/ml units per milliliter of blood. Five microliters of the spiked blood was placed on a clean plastic microscope slide (Fisher catalog #S67112A), and smeared according to the CDC described procedure (6,7). Glass was replaced by plastic slides as per our institutions biosafety protocols within the containment level 4 laboratory.

Briefly, blood was smeared using a clean plastic slide overt top of the droplet, once capillary action pulled the blood laterally, a continuous motion was used to thinly spread the blood droplet across the slide. Slides were air dried for 15 minutes in a biosafety cabinet at room temperature. Polypropylene Coplin jars (Fisher catalog # S90130) were filled with 100% methanol and slides were submerged in methanol for 15, 30 or 60 minutes, removed, and air dried for 10 minutes. A combination of chemical and heat inactivation was investigated by placing a second set of methanol fixed slides at 56°C for 1 hour utilizing a Boekel slide moat incubator (Fisher I05-450-31). Following each treatment, virus elution was performed by

*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca, 1015 Arlington St Winnipeg Manitoba R3E 3R2 204-784-7505
placing the treated slides within a 6 well tissue culture dish, and rinsed with 1 mL of Dulbecco’s Modified Essential Media (DMEM+2% FCS+10units/ml Pen/Strep), scraped for one minute using a pipette tip and titrated in VeroE6 cells (ATCC CRL 1586) using the Reed-Muench TCID₅₀ procedure (8). Two treatments of three biological replicates were assessed, each including three technical replicates.

Infectivity loss from the drying procedure was tested by comparing the fresh, non-smeared (5μL) spiked blood to the dried-smear sample. This revealed a small difference, with the undried sample yielding 5.9 (± 0.12 SD) Log TCID₅₀/mL compared to 4.7 (± 0.19 SD) Log TCID₅₀/mL for the dried sample. This loss of 1.2 logs was likely a result of the effects of drying on the virus and was considered statistically significant (Student’s paired T-test; p=0.00037).

Methanol fixation proved effective at completely inactivating the EBOV/Mak virus at all examined time points (15, 30 and 60 minutes) with no additional benefit from heat treatment at 56°C for one hour following the fixation step (Figure 1). Thus, methanol treatment alone is sufficient for EBOV inactivation and improving biosafety. Although not reported in this study, multiple reports have indicated that neither methanol fixation nor heat inactivation will negatively affect the quality of whole blood thin smears or the diagnosis of malaria during the staining procedures for malaria (9, 10, 11, 13,14).

The CDC’s guidelines state that handling of suspected EBOV cases in whole blood while screening for malaria should be treated with the addition of small amounts of Triton X-100 (6, 7, 17). Although it is believed that the additional Triton X100 would inactivate EBOV particles within malaria samples and the impact on clinical biochemical and histology assays is minimal

*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca, 1015 Arlington St Winnipeg Manitoba R3E 3R2 204-784-7505
In conclusion, a 15-minute methanol fixation step was able to inactivate a high Ebola viral load within a whole blood thin smear sample. Given the efficiency of methanol alone, it is unlikely that heat treatment would provide an additional benefit. While there was a statistically significant decrease in the viral titer from air-drying alone (1.2 logs), the resulting EBOV/Mak viral load (4.7 logs) is still considered to pose a high risk to individuals handling such slides prior to methanol fixation. It is our belief that that the handling of the thick smears for suspected Ebola infection poses a higher risk as they contain larger volumes of infected blood and are not methanol fixed and should not be used. While both the thin and thick smears allow for the detection of the *Plasmodium* parasites, the thin smears can further identify species and can accommodate the additional methanol fixation step, rendering any EBOV inert.

Methanol fixation and its application to other important high containment pathogens is currently underway. Diagnostic laboratories must therefore carefully design safe handling protocols for the preparation and handling of the malaria smear prior to the fixation step.

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*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca. 1015 Arlington St Winnipeg Manitoba R3E 3R2 204-784-7505
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*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca, 1015 Arlington St Winnipeg Manitoba R3E 3R2 204-784-7505
Figure 1. Effect of Methanol fixation against whole blood spiked with 10^8 TCID50/ml ZEBOV/Mak. Whole blood was spiked with 10^8 TCID50/ml Virions with 5 µl of spiked sample dried on microscope slide. Slides were submerged in 100% methanol for 15, 30 and 60 minutes and evaluated with an additional second set of methanol treated slides evaluated with an additional hour 56°C heat treatment. Infectious viral particles are presented with standard deviations.

*Corresponding Author. Steven.Theriault@phac-aspc.gc.ca. 1015 Arlington St Winnipeg Manitoba R3E 3R2 204-784-7505