**Background:**

Viral hemorrhagic fevers (VHFs) are a group of illnesses caused by mainly five families of viruses namely Arenaviridae, Filoviridae, Bunyaviridae, Orthonairovirus, and Paramyxoviridae (Henipavirus genus). The Filoviruses species known to cause disease in humans, Ebola virus (Zaire Ebolavirus), Sudan virus (Sudan Ebolavirus), Tschumi forest virus (Tchi Forest Ebolavirus), Bundibugyo virus (Bundibugyo Ebolavirus), and Marburg virus are restricted to Central Africa for 35 years, and spread to Guinea, Liberia, Sierra Leone in early 2014. Lassa fever is responsible for disease outbreaks in West Africa and in Southern Africa in 2008, with the identification of novel world arenavirus (Lulo virus). Henipavirus spread South Asia to Australia. COHF spread east to south europa. They are transmitted from host reservoir by direct contacts or through vectors such as ticks bits. Working with VHFs virus, need a Biosafety level 4 (BSL-4) laboratory, however during ebola outbreaks observed with Ebola virus in 2014, the need to diagnose rapidly the patients raised the necessity to develop local laboratories. The findings of this study support the concept that the standard operating procedure of the conventional approach is safe for laboratory personnel as soon as the virus is in contact with the lysis buffer after specimen injection into the self-contained pouch.

**AIM:**

An FilmArray Bio Throat-E assay for detection of Hemorrhagic fever virus such as Ebola virus was developed to respond to Hemorrhagic fever virus outbreaks.

Questions have been raised about whether the clinical sample remain biohazard after injection into the FilmArray (FA) pouch.

The FilmArray pouch contains all the required reagents for sample preparation, reverse transcription-PCR, PCR, and detection in a freeze-dried, room temperature stable format. Prior to a run, the operator injects hydration solution and the unknown sample into the pouch.

We sought to assess the biohazard of the FA Biothrot-E test by measuring the Ebola viral, Niapah viral and COHFV inactivation after exposure to the FA lysis buffer in BSA and whole blood condition.

All infectious work was performed inside a BSL-4 facility in Lyon, France.

To reproduce and assess the immediate virus-inactivating effect of the FA lysis buffer after sample injection into the FA pouch, cultured Ebola virus, Niapah and COHF were performed after 4 min contact with the FA lysis buffer, three passages were performed to ensure the inactivation efficacy.

Starting concentration of Ebola virus, Niapah virus and COHF virus were respectively of 9.55 10^5 FFU/mL, 7.44 10^5 FFU/mL, and 5.53 10^5 FFU/mL.

This study showed a rapid and total inactivation of HFV virus after contact with the FA lysis buffer. This result supports the concept that the standard operating procedure of the FA Biothrot-E test is safe for laboratory personnel as soon as the virus is in contact with the lysis buffer after specimen injection into the self-contained pouch.

**RESULTS**

Ebola virus, Niapah virus and COHF virus were passaged three times in VERO E6 cells. At first, VeroE6 cells were infected by Ebola virus, Niapah virus and COHF virus at 9.55 10^5 FFU/mL, 7.44 10^5 FFU/mL, and 5.53 10^5 FFU/mL respectively with or without treatment. Supernatants from virus-infected cells were collected at 96 hpi and used for the next passage. The P1, P2, and P3 viruses were characterized by analyzing the amount of infectious virus released into the supernatant of infected cells.

Quantities of viral RNA of VeroE6 cells infected with hemorrhagic fever viruses treated with the indicated concentrations of FA lysate + PBS or without treatment were determined by RT-qPCR.

- No viral RNA were detected at each passages after FA lysate treatment (Ct value >30).

Titers infectious virus released into the supernatant of infected cells at each passages were quantified by plaque assay in VeroE6 cells; only three experiments were performed.

- No infectious virus were detected at each passages after FA lysate Treatment (Titers < 3log).

FA Lysis activity

4 min contact with the FA lysis buffer is enough to inactivate more than 4 log of Ebola virus, Niapah and COHF virus. These results confirmed preliminary results obtained on vaccinia virus and Ebola virus.

This study showed a rapid and total inactivation of HFV virus after contact with the FA lysis buffer. This result supports the concept that the standard operating procedure of the FA Biothrot-E test is safe for laboratory personnel as soon as the virus is in contact with the lysis buffer after specimen injection into the self-contained pouch.