



Efficacy Test Results

ZOONO EBOLA SURROGATE ANTIVIRAL TEST

Mark Phelps

Zoono



ZOONO[®]

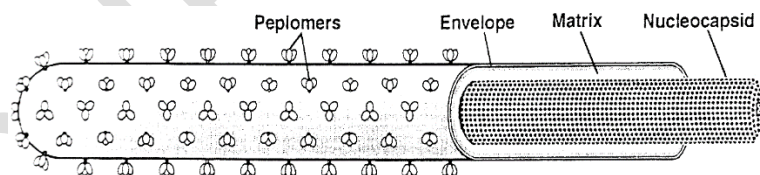
Zoono efficacy against the Ebola surrogate Respiratory Syncytial Virus (RSV)

Overview

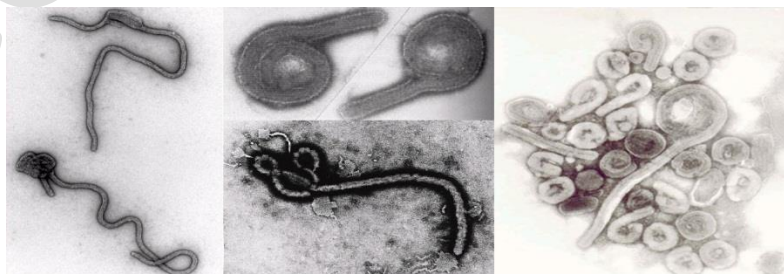
Respiratory Syncytial Virus (RSV) is recognised globally as a surrogate for the Ebola virus for the purposes of laboratory biocidal efficacy testing as it belongs to the same order and is structurally similar. This is recognised by scientists and healthcare professionals across the globe for the purpose of the evaluation of biocidal efficacy against the Ebola virus. Both Ebola and RSV belong to the order **Mononegavirales**.

Ebola virus - explained

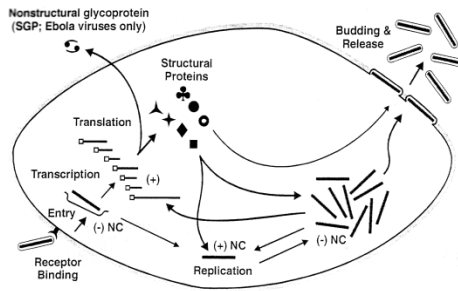
Ebola virus belongs to the Filovirus family and it is an enveloped virus. The envelope is lipid based to which antigens (peplomers) are attached. Peplomers are glycoprotein spikes that mediate the entry of the virus into the host cell through binding with cell receptors. The envelope and associated peplomers are an essential component of the virus to enable entry into the cell and replicate, without it the virus becomes inactivated and cannot initiate infection. The virus is commonly contracted from infected animals, so the infection is categorised as a zoonosis.



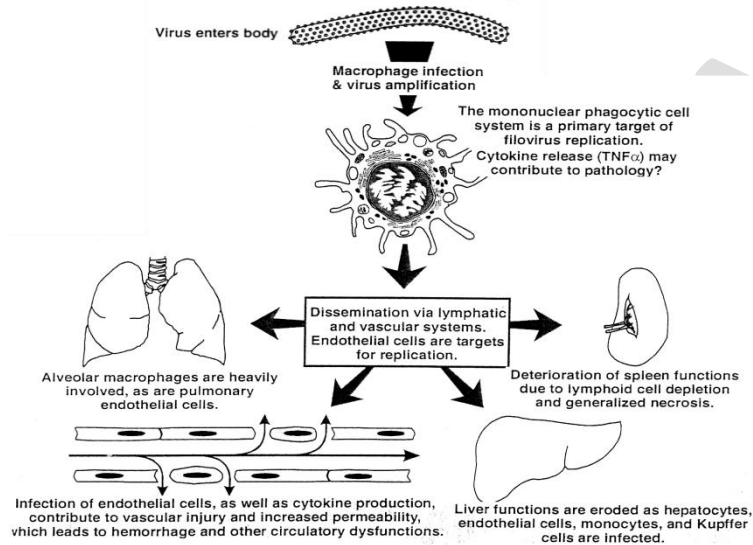
The Ebola virus is pleomorphic, meaning that it can exist in 4 distinctive shapes.



The Ebola virus enters the cell by attaching itself to the cell by binding to cell receptors with the virion spikes which then allows the RNA nucleocapsid to enter the cell to initiate the replication process.

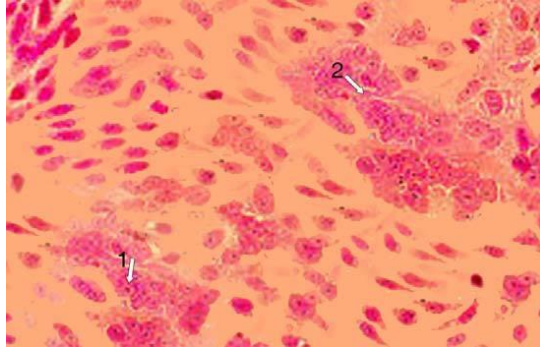


The diagram below shows the target cells and organs leading to the eventual haemorrhagic symptoms and organ failure, hence the high mortality rate.



Test overview

Antiviral biocide testing has to be carried out using tissue culture containing cells susceptible to the virus such as Hep-2 cells (Human epithelial) in order to detect the virus particles in a suspension. Unlike bacteria that can be cultivated on agar plates viruses require live cells that are grown in special tissue culture liquid (medium) and a monolayer of the cells are grown on an appropriate culture plate. The number of viruses are counted by adding a known volume of a test suspension to the tissue culture and after incubating the number of live viruses determined by their effect on the cells by microscope called, Cytopathic Effects (CPE). These are found by looking for cell abnormalities called inclusion bodies that can be seen on the photograph below and counting them which will provide the numbers of live viruses and expressed as a number of virus particles by volume. The CPE's can be visualised by staining the cells allowing the microscopic effects to be detected by the microscopist.



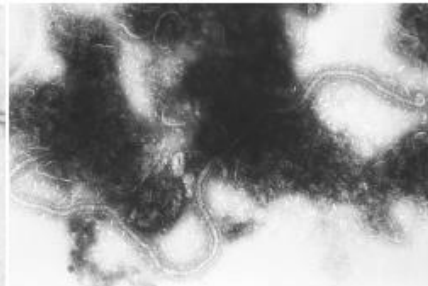
The virus suspension is then exposed to the required test concentration for a pre-determined contact time, the biocide then inactivated, and the remaining live viruses counted as described before using tissue culture, this is then expressed as a reduction.

The Respiratory Syncytial Virus is used in this test as a surrogate as it is from the same order of viruses as Ebola and safer to test in a routine laboratory.

Easton, C. R.; Pringle (2011), "Order Mononegavirales", in King, Andrew M. Q.; Adams, Michael J.; Carstens, Eric B. et al., *Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses*, London, UK: Elsevier/Academic Press, pp. 653–657, ISBN 978-0-12-384684-6



Ebola virus



RSV

Conclusion


The test sample Zoono provided was at ready to use concentration, which resulted in a 99% reduction as shown in the full test document (available on request). As the test was carried out against a virus outside the EN standard (bespoke) there is no pass or fail criteria.

Conclusion

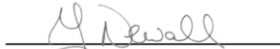
The product **Z-71 Microbe Shield** has shown a log reduction of **2.42 (99.62%)** against **Respiratory Syncytial Virus**, when tested under **clean** conditions with a contact time of **5 minutes**, at a concentration of **neat**.

See raw data tables below for test results.

The sample will be retained for 1 month unless otherwise requested.



Technical Projects Team Leader
Megan Barrett



Senior Microbiologist
Yvie Newall

CONFIDENTIAL