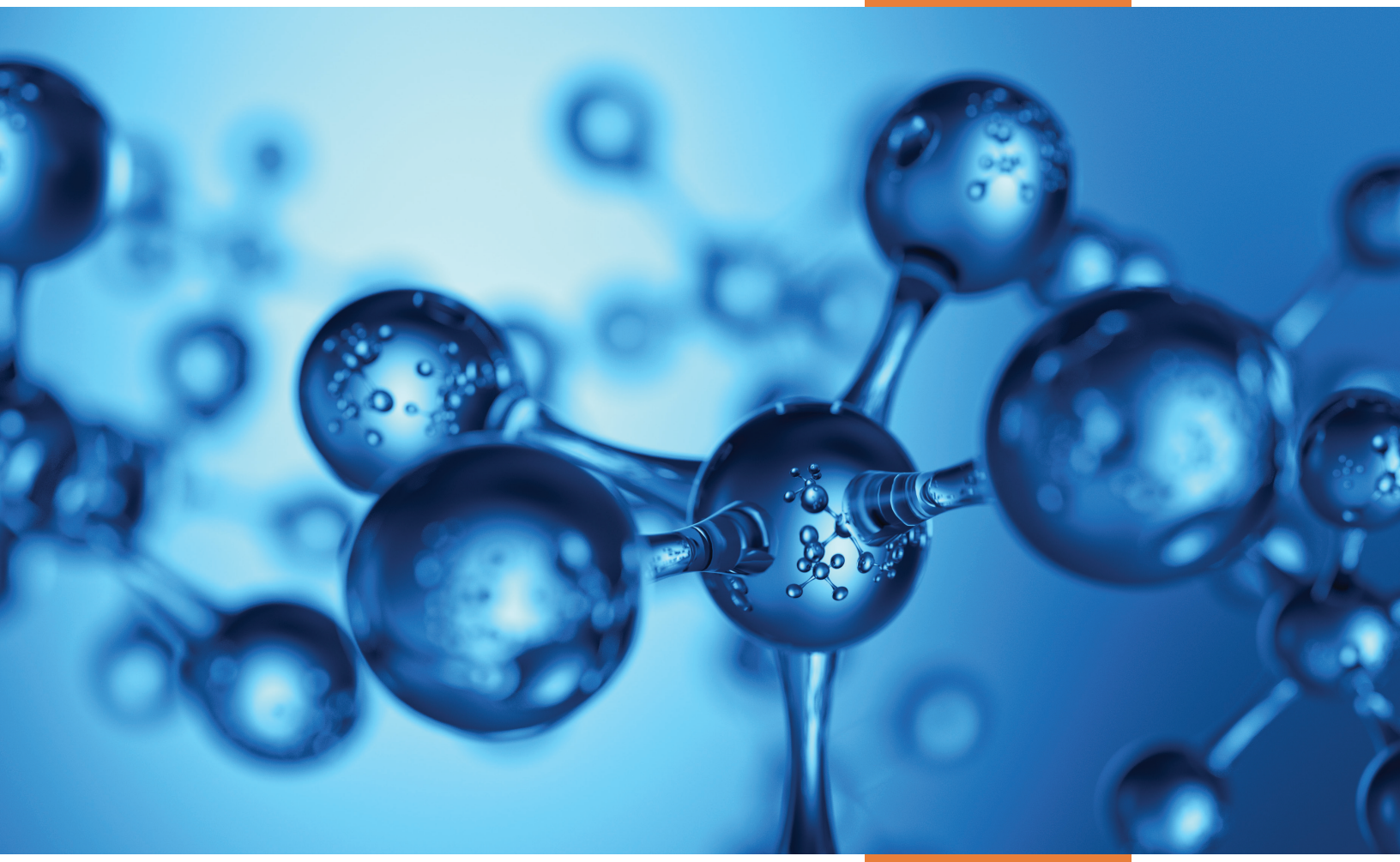
IBT Bioservices Guide to *In Vitro* Antiviral Testing



IBT BIOSERVICES

Dear Researcher,

IBT Inc. has been a pioneer in infectious disease research and continues to be on the scientific forefront of global public health initiatives. IBT Bioservices offers a variety of research services to support proof of concept, down-selection screening and activity/target validation. We use validated assays and systematic testing to shorten your time to development with greater confidence. In this document, we would like to share with you a sample of the *in vitro* antiviral assays we offer. Additional and custom services are available upon request.

We welcome your questions and feedback about the information presented here!

Sincerely,

M. Javad Aman and the IBT Bioservices Team

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In Vitro Antiviral Testing Overview

A key step in drug discovery is screening to evaluate antiviral activity. After determining the appropriate compounds and mechanisms, the next step is to perform cytotoxicity analysis of the compounds to ensure your efficacy data are meaningful and within a reasonable therapeutic window. Here is a short list of our antiviral assays.

- **Cytopathic effect (CPE) inhibition assay.** CPE is morphological changes in cells caused by cytopathogenic virus infection. CPE assay is used to evaluate test articles' ability to inhibit CPE. This is the most cost-effective and time-efficient assay we offer for high throughput screening of overall antiviral activity. For noncythopathic viruses we offer cell-based enzyme-linked immunosorbent assay (ELISA) or quantitative real-time Polymerase Chain Reaction (PCR) assay.
- **Cell-based ELISA.** Cell-based ELISA measures reduction of viral antigen in infected cells using anti-virus monoclonal antibody. The abundance of viral protein in infected cells treated with the test article compared to that of the untreated control is used as a measure of antiviral activity.
- **qPCR assay.** qPCR assay uses oligonucleotide primers and a probe amplifying virus-specific target sequence to detect the presence of virus nucleic acids. Reduction of virus nucleic acid in infected cells is used an indicator of a test article's antiviral efficacy.
- **Plaque reduction assay.** Infectious virus particles multiply in cells and result in circular zones of infected regions, plaques. Plaque reduction assay measures the plaque forming efficiency of a virus in the presence of different concentrations of a test article. Plaque reduction neutralization test (PRNT), a variation of this assay, is considered the gold standard for detecting neutralizing antibodies to certain viruses (i.e., flavivirus).
- **Yield reduction assay.** Yield reduction assay is a labor-intensive but powerful technique for evaluating a compound's antiviral efficacy. The three-step assay involves: infecting cells in the presence of different concentrations of the test article; collecting the cells or cell culture supernatants after a cycle of virus replication; and determining virus titers by plaque assay, $TCID_{50}$, or quantitative real-time PCR.
- **Antibody-dependent enhancement (ADE) assay.** ADE occurs when non-neutralizing or sub-neutralizing antiviral proteins facilitate virus entry into host cells leading to enhanced infectivity. ADE, which has been observed in viruses such as Dengue and Influenza, poses a challenge in vaccine development. Using flow cytometry, plaque assay or qPCR, this assay evaluates the ADE effect of test articles on virus infection in Fc receptor bearing cells.
- **Hemagglutination-inhibition test (HAI).** HAI assay tests the efficacy of influenza vaccine candidates in preventing virus-induced hemagglutination. We offer HAIs against H1, H7 and H10 subtypes of the Influenza virus.
- **Quantitative suspension/carrier test**. This test is used to evaluate virucidal activity of chemical disinfectants within a given contact time in suspension or on a non-porous surface. Generally, a 4 $\log_{10}$ reduction in virus titer (99.9% inactivation) is an indicator of a disinfectant's virucidal properties detected under the test conditions.

IBT Bioservices offers a set of assays in key virus families. We can help you select the appropriate assays for your specific compound and mechanism of action, as outlined in the table on the following page. Just contact us for aconsultation and a no obligation quote.

Antiviral Testing Assays

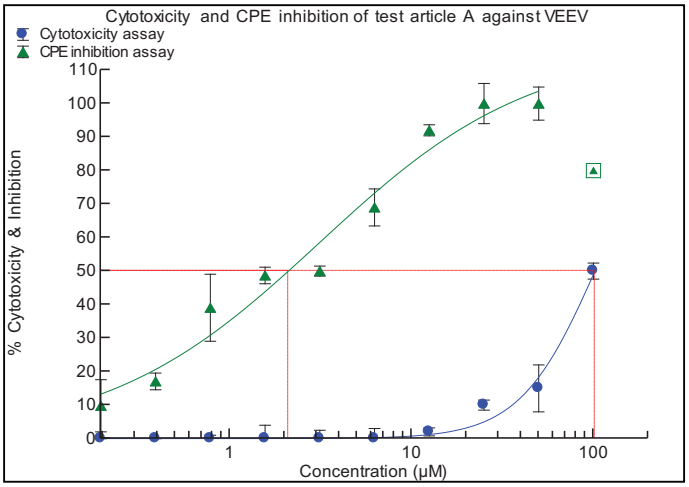
	GENUS		STRAIN
Togaviridae	Alphavirus	Chikungunya Venezuelan Equine Encephalitis	181/25 TC-83
Flaviviridae	Flavivirus	Dengue 1-4 Japanese Encephalitis Yellow Fever Zika	Various 14-14-2 17D FSS 13025
Arenaviridae	Arenavirus	Junin	Candid 1
Orthomyxoviridae	Influenza A Influenza B	H1N1, H3N2	Various Various
Bunyaviridae	Phlebovirus Orthobunyavirus	Rift Valley Fever La Crosse	MP12 H44-71017
Poxviridae	Orthopoxvirus	Vaccinia	NYCHBH
Paramyxoviridae	Pneumovirus	Human Respirator Syncytial Virus	A2
Herpesviridae	Simplex Virus	Herpes Simplex Virus	HSV1 HSV2
Picornaviridae	Enterovirus	Enterovirus 70(EV) Coxsackievirus Echovirus	J670/71 DN-19 Gregory

Alphaviruses *In Vitro*

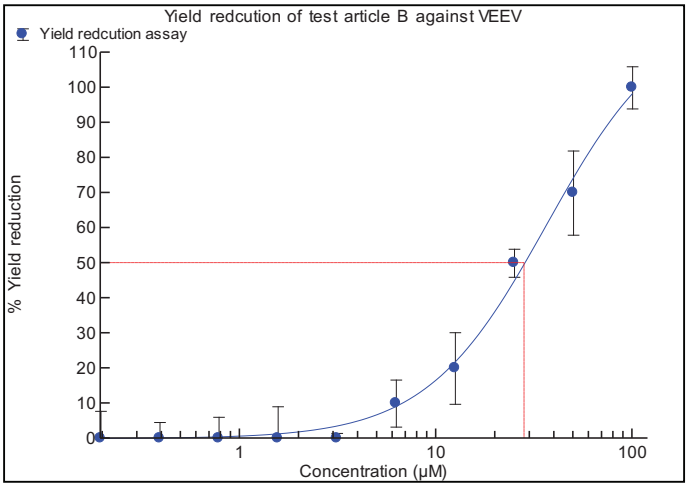
Alphaviruses are arthropod-borne viruses that cause disease in humans and a wide range of domestic and agricultural animals. Interest in the development of alphavirus therapeutics and vaccines has risen in recent years due to epidemics of Chikungunya virus (CHIKV) causing severe illness in Africa and Asia and concerns about possible use of Venezuelan equine encephalitis virus (VEEV) as an agent of bioterrorism.

A major obstacle in the development of antivirals for alphaviruses is the requirement for the work to be performed in a costly high-containment BSL-3 laboratory. IBT Bioservices has established reliable *in vitro* assays for alphaviruses using attenuated strains of CHIKV and VEEV, allowing testing in a BSL-2 environment.

Our attenuated alphaviruses include CHIKV 181/25, a well-known experimental vaccine strain, and VEEV TC-83, derived from the highly pathogenic strain Trinidad Donkey (TrD).



Vero cells infected and CPE detected using a standard crystal violet staining
Boxed point is excluded from the curve fitting of CPE assay due to the interference from cytotoxicity



Vero cells infected and cell supernatants titrated using a standard plaque assay

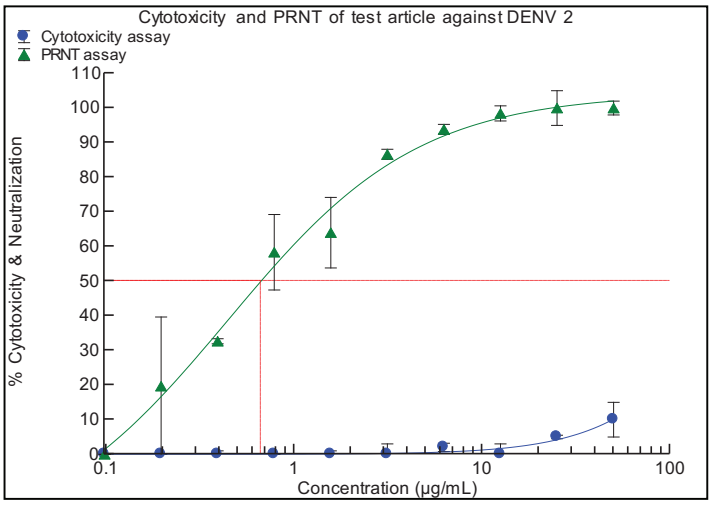
Flaviviruses *In Vitro*

Flaviviruses are a major family of pathogens and cause dangerous arthropod-borne diseases such as dengue fever (DENV), Japanese encephalitis (JEV), yellow fever (YFV), zika (ZIKV) and others.

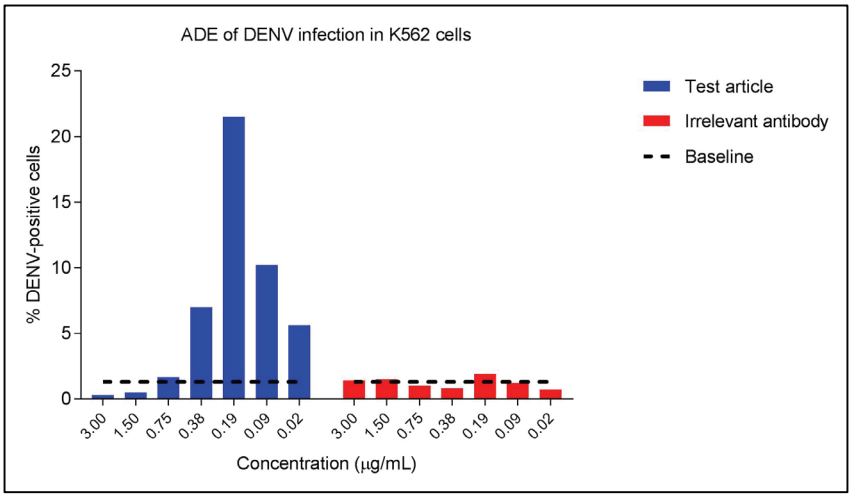
DENV infects more than 50 million people worldwide every year, causing a range of clinical syndromes from mild febrile illness to Dengue hemorrhagic fever/shock syndrome (DHF/DSS). Development of effective vaccines and therapeutics against DENV is becoming a high priority for governments and the pharmaceutical industry.

Japanese encephalitis results in high fevers and can damage the nervous system. Yellow fever is endemic in tropical regions and has a high mortality rate.

In vitro screens such as cytopathic effect (CPE), neutralization, and yield-reduction assays are used to test the activity of candidate compounds against flaviviruses. Typically, Vero cells are used for these cell-based assays. Other cell lines can be evaluated upon request.



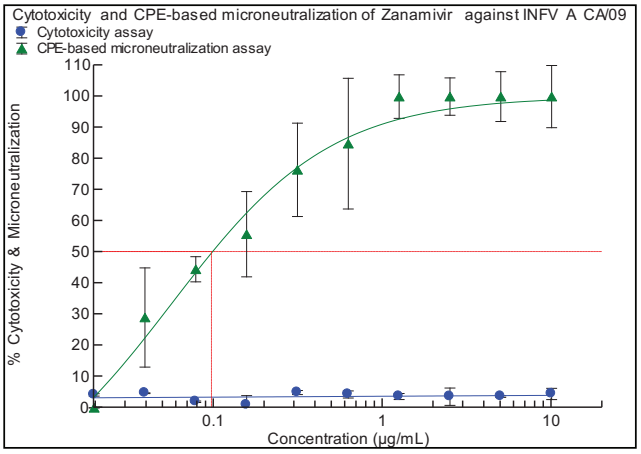
Vero cells infected and viral plaques detected using a standard immunoplaque assay



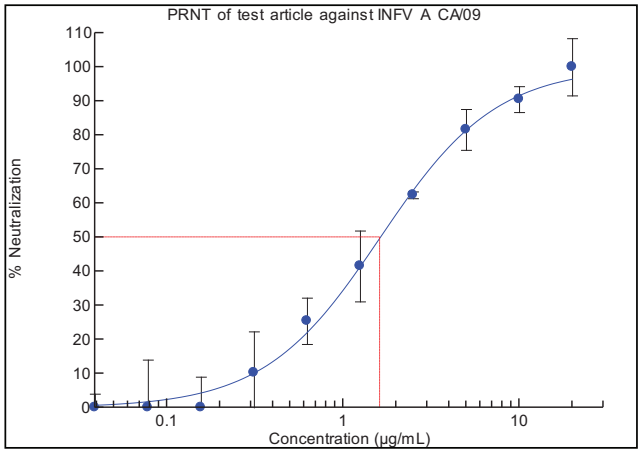
K562 cell infected and percentage of DENV-positive cells analyzed by flow cytometry

Influenza *In Vitro*

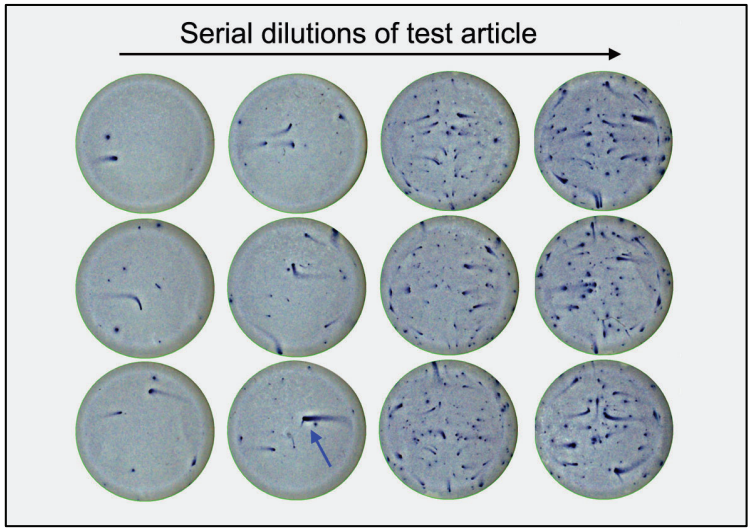
Influenza viruses (INFV) are segmented RNA viruses that cause seasonal influenza epidemics and influenza pandemics. INFV A infects birds and some mammals (e.g. pigs and humans), while INFV B only infects humans and seals. Both Influenza A and B pose a severe threat to public health and the agricultural economy. Influenza viruses mutate quickly by antigenic drift and reassortment, requiring the development and reformulation of new vaccines each year. Influenza strains resistant to almost all known drugs have been identified, making influenza drug development a high priority. Concerns about INFV have been further heightened due to the potential for transmission of highly pathogenic avian influenza (HPAI) strains to humans.



MDCK cells infected and CPE detected using a standard crystal violet staining



Vero cells infected and viral plaque detected by a standard immunoplaque assay

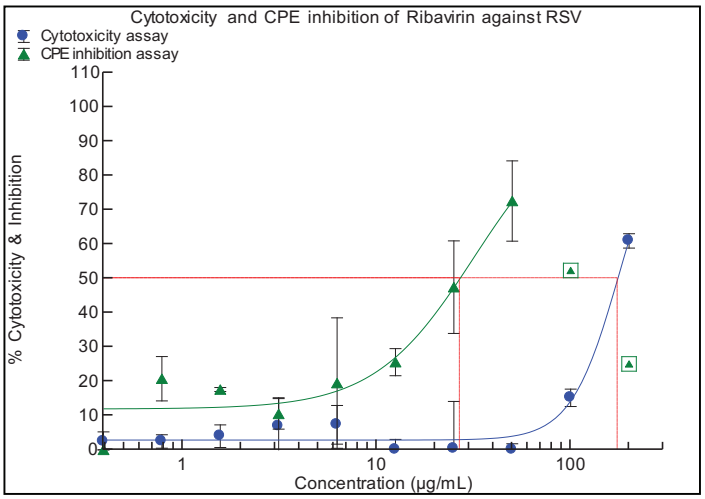


INFV plaque formation in PRNT assay.

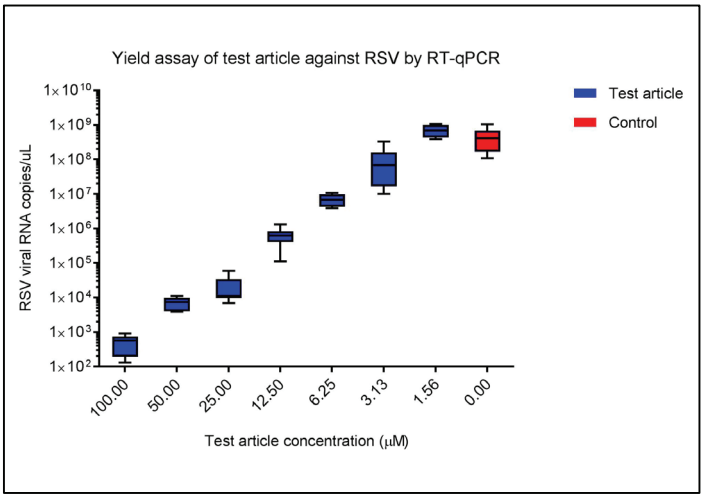
RSV *In Vitro*

Respiratory syncytial virus (RSV) is a major human pathogen causing mild to severe respiratory infections. RSV infection is particularly serious in infants and young children and causes more than 125,000 hospitalizations and 400 deaths among infants annually. Vaccine and treatment options are limited. RSV induced pathology includes damage from both the virus and the host immune response.

In vitro studies are used to test drug candidates for activity against RSV. IBT uses the RSV A2 strain in the well-established Vero and HEp-2 cell lines.



HEp-2 cells infected and CPE detected using a standard crystal violet staining
Boxed point is excluded from the curve fitting of CPE assay due to the interference from cytotoxicity

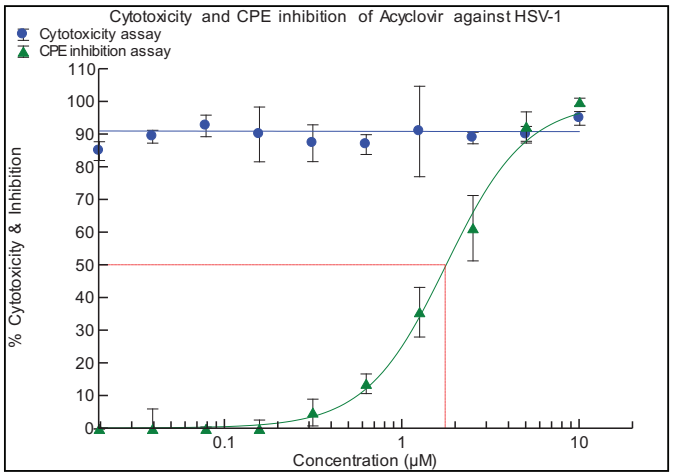


RSV viral RNA copies in yield assay of test article by RT-qPCR. Control is virus only.

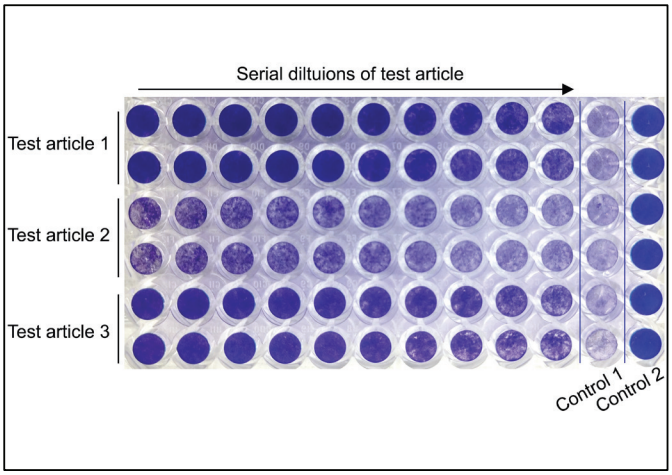
Herpes *In Vitro*

Herpes simplex virus 1 (HSV-1) is a ubiquitous and contagious virus that mainly causes cold sores and also has been found in genital infections. Herpes viruses are neurotropic and can enter a latent stage, escaping the immune system.

A majority of the US population is assumed to be latently infected with herpes viruses. Reactivation can occur sporadically resulting in efficient trasmission to new hosts. There are only a few antivirals to treat the symptoms and reduce transmission rates.



Vero cells infected and CPE detected using a standard crystal violet staining



HSV-1 CPE by crystal violet staining. Control 1 is virus only and control 2 is cells only

For more information on the services that IBTBioservices provides, please contact Haimi Shiferaw at hshiferaw@ibtbioservices.com.

IBT BIOSERVICES
4 Research Ct,
Suite 300
Rockville, MD
20850
Toll Free Phone:
(877) 411-2041
www.ibtbioservices.com

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