

Product Efficacy Overview

In vitro studies of VFI Humic Acid® have shown that the materials are efficacious against a variety of virus types, including AIDS, herpes, influenza, and hemorrhagic fevers; the data are summarized below, while the full reports are provided in the accompanying files

Inhibition of HIV Replication

USC Medical School

In vitro efficacy testing against four HIV-infected cell lines (Jurkat, HUT 78, U937, and PBMC) of one synthetic humic acid and one natural-product humic acid.

Results:

- Complete inhibition of viral replication obtained at 25 µg/mL with synthetic humic acid as well as natural-product humic acid (AZT gave comparable efficacy at 50 µg/mL, but with considerable associated cytostasis).

Southern Research Institute

In vitro efficacy testing against six HIV-infected cell lines (PBMC, CEMSS, U1, ACH2, monocyte macrophages, and HeLa CD4 LTR β-gal) of three synthetic humic acids and one natural-product humic acid.

Results:

- Protection of PBMC, monocyte macrophage, and CEMSS cells against HIV-1 infection indicates that humic acids are effective *prophylactic* (i.e., *preventative*) agents.
- Antiviral activity against chronically infected CEMSK1, U1 and ACH2 cell lines, although at higher concentrations, suggests that humic acids offer an effective *therapeutic* modality (i.e., *arrested development*) for HIV, even in latently or chronically infected cells (higher concentrations are of no concern since humic acids are essentially nontoxic *in vivo* at dosage levels in excess of 1000 µg/mL).
- Inhibition of HIV-1 viral attachment using p24 and chemiluminescence methods of endpoint detection: all four humic acids gave IC₅₀ values of between 0.01 and 0.88 µg/mL in HeLa CD4 LTR β-gal cells, confirming that humic acids prevent HIV-1 infection by blocking viral attachment to host cells.

Inhibition of HSV Infection

Specialty Laboratories

In vitro efficacy testing of three synthetic humic acids and one natural-product humic acid on African green monkey kidney cells infected with herpes simplex virus (HSV-1, HSV-2).

Results:

- Cells inoculated with 0-500 µg/mL humic acid, incubated 1 hour, then infected with virus. IC₅₀ values from 6.3 (humic acid I) to 21 µg/mL (humic acid II) (HSV-1), and 4.4 (humic acid III) to 27 µg/mL (natural-product humic acid) (HSV-2). Results indicate that humic acids are effective prophylactic agents when applied prior to contact with virus.
- Cells blended with virus and 0-500 µg/mL humic acid. IC₅₀ values from 4.5 (humic acid I) to 23 µg/mL (humic acid II) (HSV-1), and 11 (natural-product humic acid) to 17 µg/mL (humic acid III) (HSV-2). Results indicate that humic acids are effective prophylactic agents when applied immediately upon contact with virus.
- Cells infected with virus, incubated 1 hour, then 0-500 µg/mL humic acid added. IC₅₀ values from 4.0 (humic acid I) to 49 µg/mL (humic acid II) (HSV-1), and 3.4 (natural-product humic acid) to 36 µg/mL (humic acid III) (HSV-2). Results indicate that humic acids are effective therapeutic agents when applied following infection by virus.

Broad-Spectrum Antiviral Efficacy

National Institutes of Health

In vitro prophylactic efficacy testing of three synthetic humic acids and one natural-product humic acid on indicated cell lines infected with herpes, influenza, and hemorrhagic fever viruses.

Results:

- **Inhibition of proliferation of Daudi (Burkitt's lymphoma-derived) cells** by synthetic humic acid I at <0.08 µg/mL.
- **Herpes Simplex types 1 and 2** (HFF cells): IC₅₀ values from 5.0 (natural product) to 17 µg/mL (humic acid I) (HSV-1), and 2.1 (humic acid I) to 6.2 µg/mL (humic acid II) (HSV-2).
- **Human cytomegalovirus (HCMV)** (HFF cells): IC₅₀ values from 28 (humic acid I) to 81 µg/mL (humic acid II).
- **Epstein-Barr virus (EBV)** (Daudi cells): IC₅₀ values from >0.40 (humic acid I) to >50 µg/mL (natural-product humic acid).
- **Varicella Zoster virus (VZV)** (HFF cells): IC₅₀ values from

24 (humic acid I) to >100 µg/mL (humic acids II, III).

- **Influenza A (New Caledonia/20/99)(H1N1), (PR/8/34)(H1N1), (Shangdong/09/93)(H3N2), and (Sydney/05/97)(H3N2)** (MDCK cells): IC₅₀ values from 0.55 (humic acid I) to 45 µg/mL (humic acid II).
- **Influenza B (Beijing/184/93), (Harbin/07/94), and (Hong Kong/5/72)** (MDCK cells): IC₅₀ values from 0.55 (humic acid I) to 7.0 µg/mL (humic acid II).
- **Time-of-addition studies:** IC₅₀ obtained at dose levels of 5-15 µg/mL synthetic humic acids with influenza A (New Caledonia/20/99) (H1N1) at 0-1 h following viral infection, the dose levels increasing thereafter.
- **Pichende hemorrhagic-fever virus** (BSC-1 cells): IC₅₀ values <1.0 µg/mL for all humic acids.
- **Punta Toro A hemorrhagic-fever virus** (LLC-MK₂ cells): IC₅₀ values from 0.50 (natural-product humic acid) to 5.5 µg/mL (humic acid I).
- **Time-of-addition studies:** IC₅₀ obtained at dose levels of 3-40 µg/mL humic acid I with Punta Toro A virus (LLC-MK₂ cells) at 0-1 h following viral infection, the dose levels increasing thereafter.
- Overall, results confirm nontoxicity as well as broad-spectrum antiviral efficacy of humic acids, particularly against influenza and hemorrhagic-fever strains; mechanism of antiviral activity strongly indicated by time-of-addition studies to be inhibition of viral fusion.