

**Testing for Virucidal Activity
of Manorapid Synergy (Antiseptica)
vs. human rhinovirus 14 (HRV14)
and bovine viral diarrhea virus (BVDV),
a surrogate hepatitis C virus**

Scientific Report

Of the research work ordered and sponsored by Antiseptica chem.-
Pharm.prod.GmbH (Pulheim-Brauweiler, Germany)
And performed in the Stephan Angeloff Institute of Microbiology
(Department of Virology), Bulgarian Academy of Sciences (Sofia, Bulgaria)

Head of the research team: Prof. Angel S. Galabov, M.D., D.Sc.

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A SHORT DESCRIPTION OF THE VIRUCIDAL TESTING SETUP USED

The testing of the disinfectant composition MANORAPID SYNERGY (ANTISEPTICA) followed strictly the “Guidelines of Bundesgesundheitsamt (BGA) and Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (DVV) for Testing the Effectiveness of Chemical Disinfectants Against Viruses” [Zbl. Hyg. 189, 554-562 (1990)].

MANORAPID SYNERGY (ANTISEPTICA) [ManSyn] was tested for virucidal activity vs. human rhinovirus 14 (strain 1059) (HRV14) (supplied by ATCC) and bovine viral diarrhea virus (strain TVM) (BVDV), a surrogate hepatitis C virus.

The contact suspension test (standard suspension test) in vitro procedure was used for determination of virucidal activity.

MATERIALS AND METHODS

Substance tested

Manorapid Synergy was supplied by Antiseptica chem.-pharm.Prod.GmbH (Pulheim-Brauweiler, Germany) as a preparation VP-100/33A. It is a transparent colorless solution with pH 3.5.

Viruses

Human rhinovirus type 14 (strain 1059) [HRV14] (supplied by ATCC), grown in MRC-5 cells (maintenance solution DMEM Gibco BRL, Scotland, UK, plus 2% fetal calf serum). Infectious virus titer $10^{6.6}$ CCID₅₀/ml.

Bovine viral diarrhea virus (TVM strain) [BVDV], cultivated in calf trachea cell line [CT cells] (maintenance solution DMEM Gibco BRL, Scotland, UK, plus 0.5% fetal calf serum Gibco BRL, Scotland, UK); infectious titer $10^{7.0}$ CCID₅₀/ml.

Cells and Media

MRC-5 cells (human embryo lung diploid cells), supplied by ATCC) were cultivated in Costar plastic vessels (USA) with growth medium DMEM Gibco BRL, containing 10 % fetal calf serum (Gibco) and antibiotics (penicillin 100 U/ml, and streptomycin, 100µg/ml).

CT cells (calf trachea cell line) from the cell culture bank of the Stephan Angeloff Institute of Microbiology, BAS (Sofia, Bulgaria), were cultivated in Costar plastic vessels (USA) with DMEM Gibco BRL, containing 10 % fetal calf serum (Gibco) and antibiotics (penicillin 100 U/ml, and streptomycin, 100µg/ml).

Contact suspension test (standard suspension test)

The test was performed according to the “Guidelines of Bundesgesundheitsamt (BGA) and Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (DVV) for Testing the Effectiveness of Chemical Disinfectants Against Viruses”.

Contact samples. The efficacy of the disinfectant was tested in the presence of added protein in three preparations: without protein, with 0.2% serum albumin and with 10% fetal calf serum concentrations, referred to the final concentrations in the test. Virus control preparations contained the same protein concentrations.

Manorapid Synergy was tested undiluted.

Virus suspension, 2% solution of serum albumin (Sigma, USA), fetal calf serum (Gibco BRL, Scotland, UK), double-distilled water, and the respective Manorapid Synergy dilution were brought at +20°C. One part of the virus suspension was mixed with one part of the 2% serum albumin solution, or one part of fetal calf serum, or one part of double-distilled water. Then, weight parts of the diluted disinfectant having a concentration 1.25 times higher than the final test concentration were added to each preparation. The mixtures (contact samples) were kept at 20°C (in a water bath) for the exposure period to be tested (30 seconds, 1, 2, 3 and 5 minutes).

Infectious virus assay. At the end of the exposure (contact) period 10-fold serial dilutions of the contact samples were prepared in icecold culture medium (0.5 ml contact sample + 4.5 ml medium) and immediately placed into ice-water bath. Then, dilutions were inoculated into respective cell culture in suspension state, plated in 96-wells microplates (Costar, USA) and incubated at 37°C (for a time period indicated in Results). Six wells per each contact sample dilution were inoculated. Cytopathic effect was recorded microscopically and the infectious virus titer in CCID₅₀ was evaluated by the end-point dilution method of Reed and Muench.

Cytotoxicity assay. A control of the disinfectant toxicity was included as a simultaneous test of the virucidal testing: 8 parts of the diluted disinfectant were mixed with 2 parts of Dulbecco's PBS; then serial dilutions were prepared and added to the respective cell culture suspension. The effect of compound tested on cellular morphology was traced for overt signs of cytotoxicity till the end of the period of infectious virus assay recording.

Calculation of the Virucidal Effect and Statistics

All data are results of twice repeated experiments, the mean values been presented.

According to the BGA/DVV guidelines, in the contact suspension test a disinfectant solution at a particular concentration is considered as having virucidal

efficacy if within the recommended exposure period the titer of the infectious virus is reduced by at least 4.0 log₁₀

RESULTS

Virucidal Testing of ManSyn vs. human rhinovirus 14 (HRV14)

Results of experiments concerning anti-rhinovirus 14 virucidal activity of Manorapid Synergy are presented in Table 1.

Table 1. Effect of Manorapid Synergy (VP-100/33A) vs. HRV14 in the suspension test

Contact Time	Virucidal Effect ($\Delta\log_{10}$)		
	Bach + distilled water	Bach +albumin	Bach + fetal calf serum
30 seconds	>4.6	>4.6	>4.6
1 minute	>4.6	>4.6	>4.6
2 minutes	>4.6	>4.6	>4.6
3 minutes	>4.6	>4.6	>4.6
5 minutes	>4.6	>4.6	>4.6

As seen, Manorapid Synergy demonstrated a pronounced virucidal effect vs. HRV14 at all exposure times tested. Even the shortest time of 30 seconds, was enough more than 4 logs reduction of the infectious virus titer to be recorded. The presence of proteins in the contact samples did not influence the virucidal effect.

Virucidal Testing of ManSyn vs. bovine viral diarrhea virus (BVDV)
(a surrogate hepatitis C virus)

Results of experiments concerning bovine viral diarrhea virus (BVDV) virucidal activity of Manorapid Synergy are presented in Table 2.

Table 2. Effect of Manorapid Synergy (VP-100/33A) vs. BVDV in the suspension test

Contact Time	Virucidal Effect ($\Delta\log_{10}$)		
	Bach + distilled water	Bach +albumin	Bach + fetal calf serum
30 seconds	>5.4	>4.9	>4.9
1 minute	>5.4	>4.9	>4.9
2 minutes	>5.4	>4.9	>4.9

Manorapid Synergy demonstrated a pronounced virucidal effect vs. the pestivirus BVDV (a surrogate hepatitis C virus). Even the shortest time of 30 seconds, was enough for more than 4 logs reduction of the infectious virus titer. The presence of proteins in the contact samples did not influence the virucidal effect.

