

Expert Opinion

on the efficiency of

Manorapid Synergy

against

Vacciniavirus

Summary

Manorapid Synergy (formula VP100/33A) is an antiseptic used for hand disinfection. It was tested for its efficiency against Vacciniavirus ("smallpox") in a suspension test. The product showed within a contact time of 30 sec a virucidal efficiency against this virus type of ≥ 4 lg-reduction factor.

On request of Antiseptica GmbH, Pulheim "**Manorapid Synergy**" was tested for its virucidal effect in the suspension test against Vacciniavirus, strain Elstree, according to the general directions of the German society against virus diseases (Deutsche Vereinigung zur Bekämpfung der Viruserkrankungen (DVV)).

1. Introduction

The virucidal efficiency of biocides is typically evaluated in submerge test systems, like quantitative suspension tests according to AFNOR¹, ASTM², DVV³, or recent Euro-Normes⁴. Due to the good reproducibility of test results this methodology is preferably used to comparative investigations of the spectrum of efficiency or of impact factors, like temperature, application time, or interfering substances. By this way the product performance is tested against model viruses which represent by their resistance different morphological virus types.

Although virus particles which adhere to surfaces frequently are more persistent than it would be expected from suspension test, practical recommendations for application conditions are derived usually from the results when logarithmic reduction factors are ≥ 4 . Actually there is also still a lack in standardized methods for testing the virucidal effect of instrument disinfectants under application conditions.

Here we report on a liquid alcohol-based preparation for hand disinfection. In order to simulate field conditions some test assays have been charged with bovine serum albumine (BSA) or fetal calf serum (FCS), respectively. After different test intervals aliquots are withdrawn from the test assays and transferred to appropriate host cell cultures for determination of the residual titer of active virus particles. By this way the simultaneous transfer of small amounts of biocide to the cell cultures can not be prevented but this may result in toxic effects to the host cells. Thus, cytopathic effects by residual virus particles may interfere in the cell cultures with cytotoxic effects by residual biocide molecules. In cases where cytotoxic effects exceed the cytopathic effects logarithmic reduction factors are given with a “ \geq ”.

¹ AFNOR T72-181

² ASTM E 1052-96

³ Anon.: Guidelines of Bundesgesundheitsamt (BGA, German Federal Health Office) and Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (DVV, German Association for the Control of Virus Diseases) for Testing the Effectiveness of Chemical Disinfectants Against Viruses. Zbl. Hyg., 189, 554-562 (1990)

⁴ prEN 13610 and prEN 14476

2. Materials and methods

2.1 Test product

Manorapid Synergy was tested at room temperature under clean conditions and with 0.2% bovine serum albumine (BSA) or 10% fetal calf serum (FCS) (=dirty conditions), respectively.

2.2 Test virus strain and cell culture lines

As test virus Vacciniavirus, strain Elstree was used, derived from virus strain collection of DVV. Vacciniavirus is a member of the genus orthopoxvirus and therefore very near related to smallpox (variola virus).

For virus cultivation, virus titration, and cytotoxicity tests, Vero-cells were used.

The host cells were cultivated at 37.0°C in a humidified atmosphere under 5.0% CO₂. The cells were fed once per week with supplemented DMEM⁶. For propagation, once per week the cells were re-suspended by trypsin shock and transferred to fresh medium. For virus cultivation confluent monolayer culture with an age of max. 3 days were used.

2.3 Assay

The inoculum (= suspension of test virus) was prepared by centrifugation according to the methodology of the DVV-guidelines³. The virus titer of the preparations was $3,16 \times 10^9$.

The test assays were mixed in Eppendorf cups in the following way:

100 µl dest. H₂O, or BSA, or FCS, resp.

100 µl suspension of test virus

800 µl 1.20 xfold the highest application concentration of the test product
or dest. H₂O, resp.

The tests were run at room temperature.

After the test intervals aliquots of 100µl were withdrawn and immediately transferred to ice cold supplemented PBS in serial dilutions of 1:10, 1:100 etc. up to 10^{-14} . In order to detect residual virus activity pre-cultured host cells were inoculated by 6 parallels in microtiterplates. After appropriate incubation the cells were controlled for cytopathic (= active residual virus), cytotoxic effects (= artefacts by residual biocide) or complete growth (= completely inactivated virus). The different degrees of cell damage were not distinguished but only as 0% or 100% documented.

⁶ DMEM (=Dulbecco`s modification of Eagle`s medium (Fa. Biochrom, Berlin)

+ 10% FCS PAA Laboratories

+ 0,06% L-glutamine

+ 1,00% antibiotic mix, Fa. Biochrom

2.4 Calculation of logarithmic reduction factors

The virucidal effect for each test virus is calculated from the difference of ID₅₀-values in product containing assays versus PBS containing blanks. The ID₅₀-values themselves were calculated by the method of Reed and Muench.

3. Results

The original data which were read out from the microtiterplates are given in tab. 1.

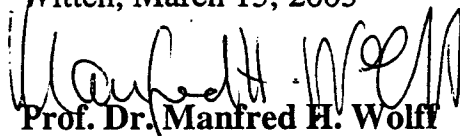
The test product has only low cytotoxic effects. This enables the calculation of the virucidal effects given in tab. 1. The virucidal effect expressed by the lg-reduction factors which result from the negative difference of ID₅₀-values in product containing assays versus PBS containing blanks.

4. Conclusion

The product Manorapid Synergy is at room temperature and as a ready for use product efficient against vacciniavirus within 30 sec application time.

The in-use dilution of the product is not susceptible to organic soil.

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Tab. 1 ID₅₀ values for Manorapid Synergy against Vacciniavirus

Concentration	Virus content in control (ID ₅₀ /ml)	Test condition	Ig reduction (log ₁₀ ID ₅₀) within minutes 0,5
ready for use product	3,16 x 10 ⁹	H ₂ O	≥ 8,5
		BSA	≥ 8,5
		FCS	≥ 8,5

H₂O = water

BSA = bovine serum albumine

FCS = fetal calf serum