

Test Procedure:

The product was tested in accordance with the "Guidelines for Testing Chemical Disinfectants for Efficacy against Viruses" drawn up by the former German Federal Health Office (BGA) and the German Association for Combating Viral Infections (DVV) - (Zbl. Hyg. 198, 554-562, 1990).

Test virus: the Mahoney-Pette poliomyelitis virus strain, supplied by the DVV for testing disinfectants, was employed. The virus was grown in Vero cell cultures, frozen 3x and used after removal of the cell debris.

The product was tested by means of the suspension test at 20°C in a water bath, with and without additional protein challenge.

Batches A, B and C were conducted for each concentration of the product.

Batch A: (1 part virus + 1 part distilled water) + 8 parts product.

Batch B: (1 part virus + 1 part 2% albumin) + 8 parts product.

Batch C: (1 part virus + 1 part fetal calf serum) + 8 parts product.

The control batches ACo, BCo and CCo were effected analogously, with 8 parts distilled water being used instead of 8 parts product.

After an exposure time of 0.5, 1, 2, 3 and 5 minutes, 0.5 ml aliquots were taken from each of batches A, B and C as well as from the control batches after a 5-minute period and were transferred to a 4.5 ml serum-free cell culture medium cooled on ice. Having completed one batch (1- 5 minutes), titration by the factor 10 (10^{-1} to 10^{-8}) was effected by pipetting 0.5 ml into the 4.5 ml medium after pipette replacement. 0.1 ml aliquots of each dilution were filled into each of 12 wells of a microtiter plate. Before testing, each well had been filled with 0.1 ml aliquots of a Vero cell suspension, containing 200000 cells/ml in a medium with 10% fetal calf serum. Once filled with the virus dilutions, the microtiter plates were incubated for 7 days at $36\pm 1^{\circ}\text{C}$ in an atmosphere with 5 % CO_2 . Microscopic counting was then conducted and statistical evaluation carried out according to the Kaerber method. The reduction factors were ascertained from the difference between the control titers and the titers obtained after exposure to the preparations.

The dilutions of the employed product were arranged such that after addition of virus and distilled water, albumin or fetal calf serum, the final dilutions of 100%, 90% and 80% were obtained. For the most vital ingredient, ethanol, final concentrations of

54.1%, 48.7% and 43.2 %
were obtained.

The test results are listed in Tables 1 and 2 as well as in Figures 1 and 2.

Manorapid Synergy (80%)

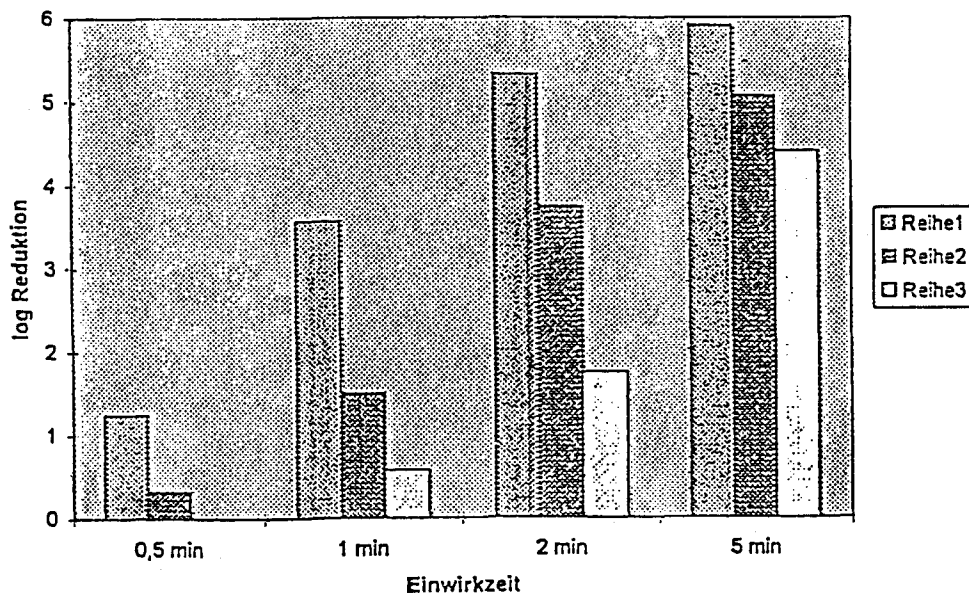


Abb 3: Inaktivierung von Poliomyelitisvirus Mahoney durch MANORAPID SYNERGY 80 %ig
Darstellung der logarithmischen Reduktionsraten nach 0,5, 1, 2 und 5 Minuten Einwirkzeit
Ansatz A(Reihe 1): ohne Belastung
Ansatz B(Reihe 2): Belastung mit 2% Albumin
Ansatz C(Reihe 3): Belastung mit 10% fötalem Kälberserum

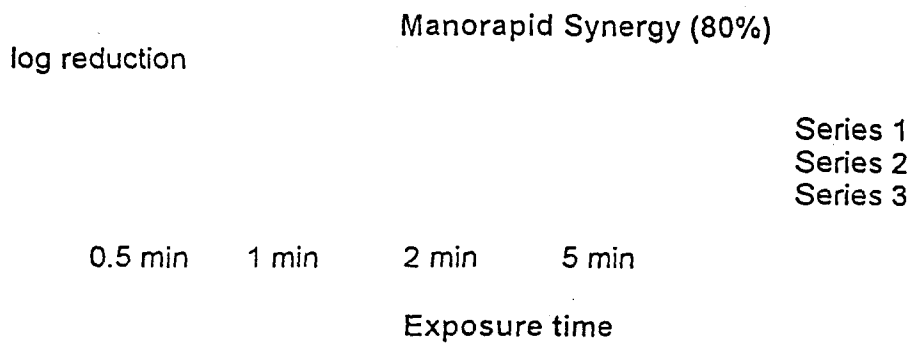


Fig 3.: Inactivation of the Mahoney poliomyelitis virus by 80% MANORAPID SYNERGY
 Illustration of the logarithmic reduction rates after 0.5, 1, 2 and 5 minutes exposure time
 Batch A (series 1): no challenge
 Batch B (series 2): challenged with 2% albumin
 Batch C (series 3): challenged with 10% fetal calf serum

Manorapid Synergy (90 %)

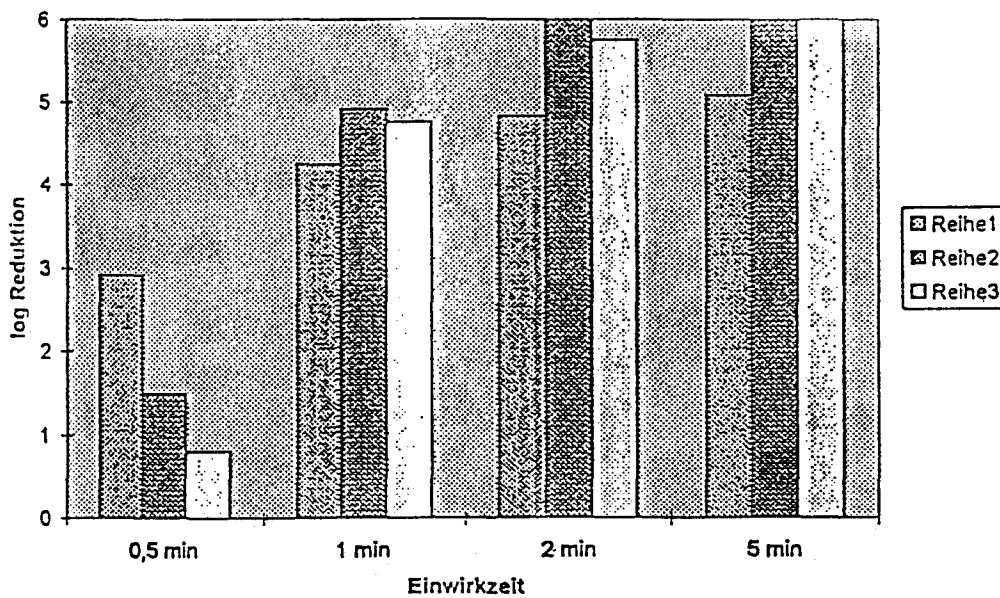


Abb 2: Inaktivierung von Poliomyelitisvirus Mahoney durch MANORAPID SYNERGY 90 %ig
Darstellung der logarithmischen Reduktionsraten nach 0,5, 1, 2 und 5 Minuten Einwirkzeit
Ansatz A(Reihe 1): ohne Belastung
Ansatz B(Reihe 2): Belastung mit 2% Albumin
Ansatz C(Reihe 3): Belastung mit 10% fötalem Kälberserum

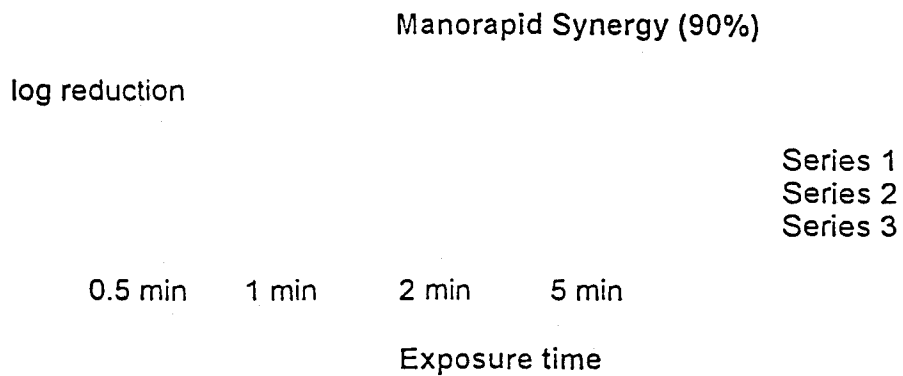


Fig 2.: Inactivation of the Mahoney poliomyelitis virus by 90% MANORAPID SYNERGY Illustration of the logarithmic reduction rates after 0.5, 1, 2 and 5 minutes exposure time

Batch A (series 1): no challenge
 Batch B (series 2): challenged with 2% albumin
 Batch C (series 3): challenged with 10% fetal calf serum

Manorapid Synergy (100 %)

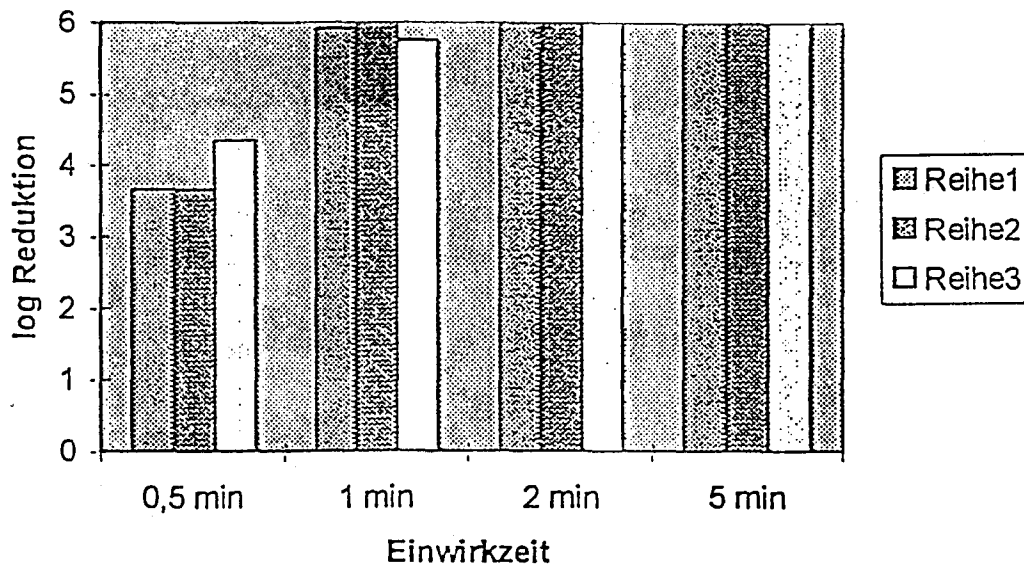


Abb 1: Inaktivierung von Poliomyelitisvirus Mahoney durch MANORAPID SYNERGY 100%ig
Darstellung der logarithmischen Reduktionsraten nach 0,5, 1, 2 und 5 Minuten Einwirkzeit
Ansatz A(Reihe 1): ohne Belastung
Ansatz B(Reihe 2): Belastung mit 2% Albumin
Ansatz C(Reihe 3): Belastung mit 10% fötalem Kälberserum

Manorapid Synergy (100%)

log reduction

Series 1
Series 2
Series 3

0.5 min 1 min 2 min 5 min

Exposure time

Fig.1: Inactivation of the Mahoney poliomyelitis virus by 100 % MANORAPID SYNERGY
Illustration of the logarithmic reduction rates after 0.5, 1, 2 and 5 minutes exposure time
Batch A (series 1): no challenge
Batch B (Series 2): challenged with 2% albumin
Batch C (series 3): challenged with 10% fetal calf serum

Table 1

Reduction Factors in the Disinfection Test with Mahoney poliomyelitis virus

Product: MANORAPID SYNERGY manufactured by Antiseptica

Evaluation after 7-day incubation period

Batch A: 8 parts product + 1 part virus + 1 part distilled water

B: 8 parts product + 1 part virus + 1 part 2% albumin

C: 8 parts product + 1 part virus + 1 part fetal calf serum

| Conc. | | 0.5 min | 1 min. | 2 min. | 5 min. |
|-----------------|---|---------|--------|--------|--------|
| 100% | A | 3.67 | 5.92 | ≥ 6 | ≥ 6 |
| (54.1% ethanol) | B | 3.67 | ≥ 6 | ≥ 6 | ≥ 6 |
| | C | 4.33 | 5.75 | ≥ 6 | ≥ 6 |
| 90% | A | 2.92 | 4.25 | 4.83 | 5.08 |
| (48.7% ethanol) | B | 1.5 | 4.92 | ≥ 6 | ≥ 6 |
| | C | 0.8 | 4.75 | 5.75 | ≥ 6 |
| 80% | A | 1.25 | 3.58 | 5.33 | ≥ 5.91 |
| 43.2 % ethanol) | B | 0.33 | 1.5 | 3.75 | 5.08 |
| | C | 0 | 0.58 | 1.75 | 4.41 |

Titers of the controls:

CoA: $10^{7.41 \pm 0.17} \text{ID}_{50}/\text{ml}$

CoB: $10^{7.25 \pm 0.13} \text{ID}_{50}/\text{ml}$

CoC: $10^{7.25 \pm 0.13} \text{ID}_{50}/\text{ml}$

Titer of virus concentrate: $10^{8.25} \text{ID}_{50}/\text{ml}$