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# Experts' Judgement

on the

virucidal efficiency

of

# *Sekusept® aktiv*

tested acc. to EN 14476

Client:: Ecolab (Dr. B. Meyer)

Authors: A. Kyas, M. Heinzel

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H e n k e l AG & Co. KGaA
VSM/Henkel - Microbiology
D-40191 Düsseldorf

Tel.: #49-211 7973458
FAX: #49-211 7982245

## Summary

*Sekusept® aktiv* is a peracetic acid (PAA)-based disinfectant used for surgical instruments. It was tested for virucidal efficiency acc. to EN 14476 (2007), i.e. a suspension test in standardized hard water against surrogate viruses serving as models for many viral pathogens, like Hepatitis virus, *Rotavirus* or *Caliciviruses*. For a sufficient efficacy this norm requires a minimum reduction in virus titer of 4.0 lg by the product. *Sekusept® aktiv* meets this requirement with a concentration of 1% in 1h or with 2% in 10 min. Thus, *Sekusept® aktiv* is virucidal in the sense of EN 14476.

## Résumé

*Sekusept® aktiv* est un désinfectant à base de l'acide peracetique (PAA) pour la désinfection des instruments de chirurgie. Son efficacité virucide a été demonstré en suspension selon la EN 14476 (2007) contre viruses posantes comme modèles pour pathogènes differents, par exemple le *Rotavirus*, les *Caliciviruses* ou les viruses de l'hepatitide. Le produit montre une reduction du titre virial de 4lg à 1% après 1 heure ou à 2% après 10 minutes. Ceci démontre ainsi l'efficacité virucidal du *Sekusept® aktiv*.

## Zusammenfassung

*Sekusept® aktiv* ist ein Peressigsäure (PAA)-basiertes Desinfektionsmittel für chirurgische Instrumente. Seine Viruswirksamkeit wurde nach EN 14476 (2007) geprüft. Dabei handelt es sich um einen Suspensionstest in standardisiertem Hartwasser gegen Simulationsviren, die als Modelle für viele virale Pathogene dienen, wie Hepatitisviren, Rotaviren oder Caliciviren. Für eine ausreichende Wirkung verlangt die Norm eine Titerreduktion um 4,0 lg-Stufen durch das Produkt. *Sekusept® aktiv* erfüllt diese Anforderung bei einer Anwendungskonzentration von 1% in 1 Stunde oder bei 2% in 10 Minuten und ist damit viruswirksam im Sinne der EN 14476.


The following experts' judgement is based on our own experimental investigations during May-August, 2008 in the facilities of VSM/Henkel-Microbiology of Henkel AG & Co. KGaA in Düsseldorf and is documented internally under the reference 08.00771.

## 1. Introduction

The virucidal efficiency of antiseptics and disinfectants is typically evaluated in-vitro by submerse test systems, like quantitative suspension tests acc. to AFNor T72-181, ASTM E1052, the German DVV & RKI[1], or the EN's 14476, 14675. Due to its good reproducibility this methodology is preferably used for comparative investigations of the efficacy or of impact factors, such as temperature, application time or interfering substances. The product performance is tested against models of non-enveloped viruses which allow for an extrapolation of the results to many other viral pathogens (cf. Annex B of the norm).

Although virus particles adhering to surfaces frequently are more persistent than would be expected from suspension tests, practical recommendations for application conditions are usually derived from these data when logarithmic reduction factors (lg RF) are ≥4.

Here we report on a powder disinfectant for surgical instruments which liberates peracetic acid (PAA) as biocidal active in a time- and temperature-depending process in aqueous solution. Therefore, the test solution was allowed to ripen for 15 minutes before the test assays were prepared. It is also advisable to prepare the use solutions in >1 liter volumes in order to prevent any inhomogeneities from the powder. The tests were performed consciously with the minimum PAA-concentrations which can be expected after 7h under storage conditions at room temperature, i.e. one shift. Under field conditions disinfectants frequently have to work under heavy duty conditions by organic loads, which may react in unspecific ways with the biocidal active. This is especially the case with oxidative actives. In order to simulate such situations, tests were charged with low or high organic load of bovine serumalbumine (BSA) and with erythrocytes which pose a strong challenge to PAA by their heme-group.

After different contact times, aliquots are withdrawn from the test assay and are transferred to appropriate host cell cultures for determination of the residual titer of infective virus particles by the formation of visible cytopathic effects (CPEs) in the host cells. The difference to the inoculum titer is the reduction factor. However, the simultaneous transfer of small amounts of biocide to the cell cultures is unavoidable with this technique and may result in cytotoxic effects (CTEs) to the detection system. In many cases CPEs differ morphologically from CTEs and thus still allow to calculate

[1] Bu.gesh.bl., 48 (2005), 1420-1426

a valid lg RF. However, in those cases where CTEs strongly exceed CPEs or when they can not be distinguished visibly, valid lg RFs can not be calculated and are given as "≥".

## 2. Materials and methodological details

The virucidal efficiency of *Sekusept® aktiv* was tested according to EN 14476 (2007). Parenthetic citations in the text (= §) refer to corresponding sections in the norm. The test conditions were stipulated by the client.

### 2.1. Test product and test conditions (§9.2.)

| | |
|---|---|
| Product name | *Sekusept® aktiv* |
| Formula-ident | 908706 |
| Intended use | Disinfection of medical instruments |
| Intended use conditions | 1%/60minutes - 2%/10minutes at room temperature |
| Batch | MD 11554-62-1 |
| Expiry date | 3/2010 |
| Manufacturer | Laboratory preparation |
| Manufacturing date / Date of delivery | Feb.2008 / April 2008 |
| Storage conditions | Room temperature |
| Biocidal active | PAA-releaser system |
| pH | 8.0 at use concentration |
| Color & smell | White powder with green speckles |
| Preparation & test concentrations | A 1.5% stock solution was prepared in 1 liter diluent and allowed to ripen for 15 minutes at room temperature. After this time the PAA content was analyzed as 1601ppm. This stock solution was diluted further with hard water into two test concentrations:<br>1) One dilution by factor 2 giving 0.75% product concentration containing 782ppm PAA per analysis. This results in a final assay concentration of 0.6% product = 625ppm PAA.<br>2) The second dilution was by factor 1.25 giving 1.2% product with 1284ppm PAA per analysis; that results in a final assay concentration of 0.96% product resp. 1025ppm PAA. The preparations were manufactured by the supplier.<br><br>600 resp. 1000ppm PAA are the minimum concentrations of biocidal active which are guaranteed by the supplier for a 1 resp. 2% use concentration after 7h. |
| Diluent | Hard water (§5.2.2.2.) |
| Test temperature | 20±1°C |
| Contact time | 10min (for 0.96% assay conc.) resp.<br>60min (for 0.6% assay conc.) |
| Annulment of contact | Direct serial dilutions in ice-cold DMEM[2] |
| Low organic load | 0.03% BSA |
| High organic load | 0.3% BSA + 0.3% erythrocytes (§5.2.3.3.3.) |

## 2.2. Reagents & material

| Reagent | Supplier | Batch |
|---|---|---|
| Blood (Sheep) | Oxoid | 964590000 exp. 2008-05-13 |
| Bovine serumalbumine | Serva | 17363 exp. 12/2008 |
| DMEM culture medium[2] | Biochrom | 0231L exp. 9/2008 |
| Fetal calf serum (FCS) | Gibco | 0647H exp. 6/2010 |
| Formol (as 36.5% formaldehyde) | Riedel-deHäen | 33220 exp. 3/2012 |
| Phosphate-buffered saline<br>(D-PBS)<br>(10x-PBS) | <br>Gibco<br>Gibco | <br>318271 exp. 9/2009<br>13216 exp. 10/2008 |
| Trypsine/EDTA | Gibco | 311776 exp. 3/2009 |
| Aqua dest. ster. | Self preparation & autoclavation | 20080 306101, fresh usage |
| Hard water solution A (§5.2.2.2.) | Self preparation | 20080 211101, fresh usage |
| Hard water solution B (§5.2.2.2.) | Self preparation | 20080 211111, fresh usage |

## 2.3. Test sytems and host cells

| Test virus | Acronym | Type strain | ICTV-index (2006) | Structure | Supply |
|---|---|---|---|---|---|
| Human *Adenovirus C* | HAdVC | Type 5 St. Adenoid 75 | 00.001.0.01.010.00.105 | Isometric, non-enveloped, dsDNA | ATCC VR-767 (2003) |
| Human *Poliovirus* | HPV-1 | Type 1 Strain LSC-2ab | 00.052.0.01.007.00.001 | Icosahedric, non-enveloped, ssRNA | Eurovir (2003) |

The virus strains were propagated in >90% confluent monolayers: HAdVC in H.Ep#2-cells and HPV-1 in RD-cells. After formation of CPEs virus was harvested by centrifugation of cell debris at 4000rpm/10min. followed by clearance filtration via 0.2µm. Aliquot parts were frozen at –70°C until usage. The vials were thawed immediately before use and virus titers were determined actually during the test.

| Cell lines | Source | Passage | DMEM + supplement |
|---|---|---|---|
| H.Ep #2 | Prof. Lindl, LMU München | 53 as of 2008-05-08 | 5% FCS |
| RD | Dr. Steinmann, Hyg.-Inst., Bremen | 25 as of 2008-05-08 | 5% FCS |

The cells were grown at 36.5°C under 8.5% $CO_2$ in a $CO_2$-incubator and transferred to 30°C when >90% confluent. Medium was replaced weekly.

## 2.4. Efficacy test (§6.6.1. – 6.6.3.)

All assay ingredients were allowed to adjust to test temperature and the assays were performed in a temperature-controlled water bath:

100µl of 10x low resp. high organic load
(→ 0.03% BSA resp. 0.3% BSA+0.3% erythrocytes in the final assays)
800µl of 0.75 resp. 1.2% test product (cf. 2.1.), resp. diluent as blank
100µl of virus inoculum preparation (= start)

Due to the dilution factor 1.25x of the test assay the final product concentrations result as 0.6 resp. 0.96%. In the raw data (tables 1-5) the concentrations are always referred to these assay concentration.

For determination of the virus inoculum titers and stability, blanks are run for 0 resp. 60 minutes with diluent instead of test product (§6.6.8.).

[2] DMEM = *Dulbecco*'s modification of *Eagle*'s medium + 5% FCS

After the contact times the reaction is stopped by transfer of 100µl aliquots to 900µl ice-cold supplemented DMEM and serial 1:10-dilutions up to $10^9$. As annulment verification this is already controlled at time 0 (cf. here 2.5.3.). The recovery of residual, infective virus was performed in quantal tests on the corresponding host cells in microtiter plates using 8 parallels each by transferring 100µl aliquots of each dilution to confluent monolayer cells (§6.5.1.2.). Incubation follows conditions given here in 2.3.

After 9 (HAdVC) resp. 4 (HPV-1) days the cultures are read out for cytopathic effects (CPEs) by an inverse microscope. The effects are evaluated as follows:

0 = no cell damage = no virus activity
1 <25% cell damage = virus activity
2 ≈50% cell damage = virus activity
3 ≈75% cell damage = virus activity
4 ≈100% cell damage = virus activity

## 2.5. Validation controls

2.5.1. Cytotoxicity controls (§6.6.4.1.)

200µl diluent
800µl test product in 1.25x test concentration (cf. 2.4.)

After mixing and direct serial 1:10-dilutions in ice-cold supplemented DMEM monolayered host cells in microtiter plates are inoculated by 100µl aliquots in 8 parallels. Incubation follows conditions given here in 2.3. In the raw data the concentrations are always referred to the final 1.0x assay concentration.

After 9 resp. 4 days the cultures are read out for cytotoxic effects (CTEs) by an inverse microscope. The effects observed could be distinguished clearly from CPEs and are evaluated as follows:

0 = no CTE;
X = CTE.

2.5.2. Interference controls (§6.6.4.2.)

100µl of 1:100-dilution of the test product resp. 100µl PBS as control are transferred to

a.) 8 parallels to monolayered host cells in microtiter plates and
b.) 100µl host cell preparations.

After 1h contact time at 37°C the supernatant is discarded and replaced by 100µl PBS-diluted virus inoculum preparation in dilution steps $10^2 - 10^9$. After another hour an additional volume of 100µl cell culture medium is pipetted in each well. Incubation follows conditions given here in 2.3.

After 9 resp. 4 days the cultures are read out for cytopathic effects (CPEs) by an inverse microscope. The effects are evaluated as in 2.4.

2.5.3. Annulment controls (§6.6.6.1.)

These controls serve for proving sufficient virus activity at start conditions of the test: From the complete test assay (cf. here 2.4.) a 100µl aliquot is to be withdrawn already at time 0±5sec. and immediately transferred to ice-cold, supplemented DMEM. These controls shall be further processed in analogy to 2.4. and the virus titers may only differ from the inoculum preparation titer by ≤0.5lg.

As shown in the raw data (cf. tables 4.1.+4.2.) this could be successfully demonstrated only in one case for *Poliovirus*. With *Adenovirus* there was already a sharp decrease in virus infectivity of >4lg which is clearly too high but which could be reproduced repeatedly. In order to verify that this effect is product-specific and no methodological artifact this annulment control was repeated with increasing product concentrations. Thus, it could be demonstrated

that it is indeed an effect of reaction kinetics and may not be due to any mistakes in the test performance.

2.5.4. Formaldehyde controls (§6.6.7.)

100µl virus inoculum preparation
400µl PBS
500µl 3.8% solution* of formalin (= 36.5% formaldehyde)
*the actual concentration of this solution was analyzed as 1.4% (w/v) formaldehyde

After contact times of 5 – 15 – 30 – 60 minutes 100µl aliquots are withdrawn and transferred to ice-cold supplemented DMEM and serial 1:10 dilutions are prepared. 100µl aliquots of each dilution are plated on monolayered host cells in microtiter plates and incubated as given in 2.3.

## 2.6. Calculation of virus titers

From the qualitative results of the residual infectivity the titer **m** of infective virus particles in the original 100µl aliquots may be re-calculated as 50% infective dose ($TCID_{50}$) by the method of *Spearman & Kärber* by the following formula:

$$\mathbf{m}\ (TCID_{50}) = \mathbf{V + [(P_v + P_{v+1} - 0.5]}$$

**m** = lg $TCID_{50}$ in first 100µl aliquot
**V** = lg of the highest dilution in which all or almost all of 8 parallels are positive (1-4)
$\mathbf{P_i}$ = quotient of positives in one series:

| Positives | $P_i$ |
|---|---|
| 0/8 | 0.000 |
| 1/8 | 0.125 |
| 2/8 | 0.250 |
| 3/8 | 0.375 |
| 4/8 | 0.500 |
| 5/8 | 0.625 |
| 6/8 | 0.750 |
| 7/8 | 0.875 |
| 8/8 | 1.000 |

Only those dilutions may be used for RF-calculations, where in the corresponding interference controls either

# the interference controls indicate titer difference of <1lg in the PBS-treated cells vs. the product-treated cells

or

# the CPEs are evaluated with 0 or 1.

The EN 14476 does not require statistical analyses of the results but in this case it seems advisable to consider the virucidal effects, i.e. the RF's, with respect to the confidence intervals (cf. 3.2. Results & Discussion):
The standard deviation $\mathbf{S_m}$ is calculated following the principle formula

$$S_m = \sqrt{1\ \Sigma\ [P_i \times (1 - P_i) : 7]}$$

In most cases, however, $P_v = 1$ and $P_{v+2} = 0$; then

$$\mathbf{S_m = \sqrt{[P_{v+1} \times (1 - P_{v+1}) : 7]}}$$

## 2.7. Calculation of the virucidal effect

The virucidal effect of the test product is calculated as reduction factor **RF**, i.e. the difference between the virus titers of the inocula (= grey highlighting) minus the virus titers found for the product-containing assays (= blue highlighting):

$$RF = lg\ (inoculum\ CPE) - lg\ (test\text{-}product\ CPE)$$

When CTEs exceed CPEs the RF is given as "≥".
The confidence intervals $K_{RF}$ of the RF-values are calculated following the formula:

$$K_{RF} = \sqrt{[(2S_m)^2 + (2S_m)^2]}$$

Following the norm the virucidal effect of a product is sufficient when the RF is ≥4lg.

# 3. Results

## 3.1. Verifications acc. to § 8.3c-e of the norm

a) The inoculum titer of *Poliovirus* was $10^8$/100µl which is 10fold higher than the norm requires. For *Adenovirus* the titer was only $10^{6.5}$/100µl which is slightly below the norm recommendation but still sufficient to verify virucidal effects of >4lg.

b) For H.Ep.#2-cells the cytotoxicity is between 0.005≤0.006% and for RD-cells ≤0.006% (cf. tables 1 & 4.1./4.2.).

c) *Poliovirus* was inactivated by the formaldehyde-standard by 2.25lg/30min. resp. >3.3lg/60min. thus showing a resistance which fits exactly in the range required by the norm (cf. tab.3).

d) In the interference controls (cf. tab.2) the titers on the PBS-treated cells differ from those of product treated cells only by ≤0.125lg which is in the norm tolerance of <1lg.

e) At the use concentrations the annulment controls didn't meet the norm requirement of Δ ≤0.5lg. We verified the following differences which fulfill only in one case the norm requirement:

TS 1.2.7.

| Virus | Concentration | Protein load | Δ (titer – annulment) |
|---|---|---|---|
| *Adenovirus* | 0.60% | low | >4 |
| | | high | >4 |
| | 0.96% | low | >4 |
| | | high | >4 |
| *Poliovirus* | 0.60% | low | 1.000 |
| | | high | 1.250 |
| | 0.96% | low | 0.250 |
| | | high | 1.125 |

Therefore, we additionally tested *Adenovirus* (which is the more susceptible of both test viruses) also in lower concentrations between 0.05-0.50%, which is presumably non-virucidal, and could thus demonstrate that this is an effect which strongly depends from the product concentration and is not caused by any failure in the test

systems (i.e. the host cells or the virus strains). Obviously the oxidative reaction effects so rapidly that the product is virucidal already within the first seconds of contact.

### 3.2. Discussion of efficacy results

Our results of the efficacy tests (cf. tabs. 4.1./4.2.) may be summarized as follows:

| Virus | Product concentration | Time (min.) | Protein load | lg RF ± $K_{RF}$ |
|---|---|---|---|---|
| *Adenovirus* | 0.60% | 60 | low | ≥4.0 ± 0 |
| | | | high | ≥4.0 ± 0 |
| | 0.96% | 10 | low | ≥4.0 ± 0 |
| | | | high | ≥4.0 ± 0 |
| *Poliovirus* | 0.60% | 60 | low | 5.375 ± 0.364 |
| | | | high | ≥5.5 ± 0.376 |
| | 0.96% | 10 | low | 4.375 ± 0.364 |
| | | | high | 3.750 ± 0.496 |

Under dirty conditions, i.e. with high challenge of protein and blood, the short time effect (10min/0.96%) shows only a RF of 3.75lg which fails the norm requirement of ≥4lg. However, considering the confidence interval of 0.5lg the effect slightly would meet the 4lg-requirement based on an acceptable tolerance of ½$K_{RF}$ (= 0.25lg in this case). These results were found with the minimum biocidal concentrations to be expected after one shift storage conditions at room temperature, thus respecting already a safety margin.

**Therefore, we come to the conclusion that for the intended use concentrations *Sekusept® aktiv* is virucidal in the sense of EN 14476 with 1%/1h or 2%/10min.**

Düsseldorf, 2008-08-12

A. Kyas

M. Heinzel

Distribution list: Ecolab (Dr. B. Meyer, Dr. F.vRheinbaben), Dr. Stelter, authors

**Tab. 1 Raw data of cytotoxicity controls 2.5.1. of *Sekusept® aktiv***

| Assay | Assay concentration | Host cell | lg dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Cell control |
| Product | 0.6% | H.Ep #2 | n.d. | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 |
| Product | 0.96% | | n.d. | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 |
| Product | 0.6% | RD | n.d. | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 |
| Product | 0.96% | | n.d. | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 | 00000000 |

**Tab. 2 Raw data interference controls 2.5.2. of *Sekusept® aktiv***

| Virus Host cell culture | Assay conc. | Cell treatment 1h/37°C | lg dilution | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | m |
| *Adenovirus* H.Ep #2 -monolayers | 0.006% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 40444440 | 00000000 | 00000000 | 6.250 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444440 | 00000000 | 00000000 | 6.375 |
| | 0.0096% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 00000000 | 00000000 | 6.500 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444440 | 00000000 | 00000000 | 6.375 |
| *Adenovirus* H.Ep #2 susp. cells | 0.006% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444440 | 00000000 | 00000000 | 6.375 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 00000000 | 00000000 | 6.500 |
| | 0.0096% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444440 | 00000000 | 00000000 | 6.375 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 00000000 | 00000000 | 6.500 |
| *Poliovirus* RD-monolayers | 0.006% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44400000 | 7.875 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44400000 | 7.875 |
| | 0.0096% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44440000 | 8.000 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44400000 | 7.875 |
| *Poliovirus* RD susp. cells | 0.006% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44440000 | 8.000 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44400000 | 7.875 |
| | 0.0096% | Product | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44000000 | 7.750 |
| | | PBS | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44400000 | 7.875 |

**Tab. 3 Raw data 0.7% formaldehyde controls 2.5.4.**

| Virus | Time (min) | lg dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | m |
| *Adenovirus* | 5 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | ≤4.5 |
| | 15 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | ≤4.5 |
| | 30 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | ≤4.5 |
| | 60 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | ≤4.5 |
| *Poliovirus* | 5 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 44444444 | 44444444 | 40000000 | 00000000 | 6.625 |
| | 15 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 44444444 | 44444444 | 00000000 | 00000000 | 6.500 |
| | 30 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 44444444 | 40000000 | 00000000 | 00000000 | 5.625 |
| | 60 | n.d. | xxxxxxxx | xxxxxxxx | xxxxxxxx | 00000000 | 00000000 | 00000000 | 00000000 | ≤4.5 |

**Tab. 4.1. Raw data efficacy *Sekusept® aktiv* under low organic load (i.e. 0.03% BSA)**

| Virus | | Assay conc. | t (min.) | Pi at lg dilution | | | | | | | | m ± $S_m$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| *Adenovirus* | Titer controls | 0 | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.500 ± 0** |
| | | 0 | 60 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.500 ±0** |
| | Annullment controls | 0.05% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 200000000 Pi = 0.125 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.625 ± 0.125** |
| | | 0.10% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 200000000 Pi = 0.125 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.625 ± 0.125** |
| | | 0.50% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 22211222 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **4.500 ± 0** |
| | | 0.60% | 0 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 0 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | Test | 0.60% | 60 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 10 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| *Poliovirus* | Titer controls | 0 | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44400000 Pi = 0.375 | 00000000 Pi = 0 | **7.875 ±0.182** |
| | | 0 | 60 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44440000 Pi = 0.500 | 00000000 Pi = 0 | **8.000 ±0.188** |
| | Annullment controls | 0.60% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44400000 Pi = 0.375 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.875 ±0.182** |
| | | 0.96% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 40000000 Pi = 0.125 | 00000000 Pi = 0 | **7.625 ± 0** |
| | Test | 0.60% | 60 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 10 | xxxxxxxx | 44444444 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **3.500 ±0** |

**Tab. 4.2. Raw data efficacy *Sekusept® aktiv* under high organic load (i.e. 0.3% BSA + 0.3% erythrocytes)**

| Virus | | Assay conc. | t (min.) | Pi at lg dilution | | | | | | | | m ± $S_m$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| *Adenovirus* | Titer controls | 0 | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.500 ± 0** |
| | | 0 | 60 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.500 ±0** |
| | Annullment controls | 0.05% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 300000000 Pi = 0.125 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.625 ± 0.125** |
| | | 0.10% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 400000000 Pi = 0.125 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.625 ± 0.125** |
| | | 0.50% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 22242222 Pi = 1.0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **4.500 ± 0** |
| | | 0.60% | 0 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 0 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | Test | 0.60% | 60 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 10 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| *Poliovirus* | Titer controls | 0 | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44440000 Pi = 0.500 | 00000000 Pi = 0 | **8.000 ±0.188** |
| | | 0 | 60 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44440000 Pi = 0.500 | 00000000 Pi = 0 | **8.000 ±0.188** |
| | Annullment controls | 0.60% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44000000 Pi = 0.250 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.750 ±0.163** |
| | | 0.96% | 0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44444444 Pi = 1.0 | 44400000 Pi = 0.375 | 00000000 Pi = 0 | 00000000 Pi = 0 | **6.875 ±0.182** |
| | Test | 0.60% | 60 | xxxxxxxx | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **≤2.5 ± 0** |
| | | 0.96% | 10 | xxxxxxxx | 44444444 Pi = 1.0 | 44222200 Pi = 0.750 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | 00000000 Pi = 0 | **4.250 ±0.163** |