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**VSM / Henkel - Microbiology**



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## **Experts' Judgement**

on the

virucidal efficiency

of

***Sekusept<sup>®</sup> aktiv***

tested acc. to EN 14476

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## **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 peracétique (PAA) pour la désinfection des instruments de chirurgie. Son efficacité virucide a été démontré en suspension selon la EN 14476 (2007) contre virus posantes comme modèles pour pathogènes différents, par exemple le *Rotavirus*, les *Caliciviruses* ou les virus de l'hépatite. Le produit montre une réduction 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 submerge test systems, like quantitative suspension tests acc. to AFNor T72-181, ASTM E1052, the German DVV & RKI<sup>1</sup>, 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  $\geq 4$ .

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

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<sup>1</sup> Bu.gesh.bl., 48 (2005), 1420-1426

a valid Ig RF. However, in those cases where CTEs strongly exceed CPEs or when they can not be distinguished visibly, valid Ig 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	<i>Sekusept® aktiv</i>
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	<p>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:</p> <p>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.</p> <p>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.</p> <p>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.</p>
Diluent	Hard water (§5.2.2.2.)
Test temperature	20±1°C
Contact time	10min (for 0.96% assay conc.) resp. 60min (for 0.6% assay conc.)
Annulment of contact	Direct serial dilutions in ice-cold DMEM <sup>2</sup>
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 <sup>2</sup>	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 (D-PBS) (10x-PBS)	Gibco	318271 exp. 9/2009
	Gibco	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 systems 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<sub>2</sub> in a CO<sub>2</sub>-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.).

<sup>2</sup> 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<sup>9</sup>. 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 (HAAdVC) 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<sup>2</sup> – 10<sup>9</sup>. 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<sub>50</sub>) by the method of *Spearman & Kärber* by the following formula:

$$m \text{ (TCID}_{50}\text{)} = V + [(P_v + P_{v+1} - 0.5)]$$

**m** = lg TCID<sub>50</sub> in first 100µl aliquot

**V** = lg of the highest dilution in which all or almost all of 8 parallels are positive (1-4)

**P<sub>i</sub>** = quotient of positives in one series:

Positives	P <sub>i</sub>
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 **S<sub>m</sub>** is calculated following the principle formula

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

In most cases, however, P<sub>v</sub> = 1 and P<sub>v+2</sub> = 0; then

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

#### 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):

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

When CTEs exceed CPEs the RF is given as “≥”.

The confidence intervals  $K_{RF}$  of the RF-values are calculated following the formula:

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

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  $10^8/100\mu\text{l}$  which is 10fold higher than the norm requires. For *Adenovirus* the titer was only  $10^{6.5}/100\mu\text{l}$  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  $0.005 \leq 0.006\%$  and for RD-cells  $\leq 0.006\%$  (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  $\leq 0.125\text{lg}$  which is in the norm tolerance of <1lg.

e) At the use concentrations the annulment controls didn't meet the norm requirement of  $\Delta \leq 0.5\text{lg}$ . We verified the following differences which fulfill only in one case the norm requirement:

Virus	Concentration	Protein load	$\Delta$ (titer – annulment)
<i>Adenovirus</i>	0.60%	low	>4
		high	>4
	0.96%	low	>4
		high	>4
<i>Poliovirus</i>	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	Ig RF $\pm K_{RF}$
<i>Adenovirus</i>	0.60%	60	low	$\geq 4.0 \pm 0$
			high	$\geq 4.0 \pm 0$
	0.96%	10	low	$\geq 4.0 \pm 0$
			high	$\geq 4.0 \pm 0$
<i>Poliovirus</i>	0.60%	60	low	$5.375 \pm 0.364$
			high	$\geq 5.5 \pm 0.376$
	0.96%	10	low	$4.375 \pm 0.364$
			high	$3.750 \pm 0.496$

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  $\geq 4$ lg. However, considering the confidence interval of 0.5lg the effect slightly would meet the 4lg-requirement based on an acceptable tolerance of  $\frac{1}{2}K_{RF}$  (= 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*<sup>®</sup> aktiv is virucidal in the sense of EN 14476 with 1%/1h or 2%/10min.**

Düsseldorf, 2008-08-12

  
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M. Heinzel

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



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

Virus	Time (min)	lg dilution								m				
		1	2	3	4	5	6	7	8					
<i>Adenovirus</i>	5	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤4.5
	15	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤4.5
	30	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤4.5
	60	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤4.5
<i>Poliovirus</i>	5	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	44444444	44444444	44444444	44444444	40000000	00000000	00000000	6.625
	15	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	44444444	44444444	44444444	44444444	00000000	00000000	00000000	6.500
	30	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	44444444	44444444	40000000	00000000	00000000	00000000	00000000	5.625
	60	n.d.	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	00000000	00000000	00000000	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 <sub>m</sub>	
			2	3	4	5	6	7	8	9				
Adenovirus	0	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	00000000	00000000	00000000	6.500 ± 0.
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0	Pi = 0	Pi = 0
	0	60	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	00000000	00000000	00000000	6.500 ± 0.
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0	Pi = 0	± 0.
	0.05%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	20000000	00000000	00000000	6.625 ± 0.125
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0.125	Pi = 0	± 0.125
	0.10%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	20000000	00000000	00000000	6.625 ± 0.125
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0.125	Pi = 0	Pi = 0	± 0.125
	0.50%	0	44444444	44444444	22212222	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	4.500 ± 0
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0	Pi = 0	Pi = 0	± 0					
0.60%	0	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	± 0	
0.96%	0	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	± 0	
0.60%	60	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	± 0	
0.96%	10	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	± 0	
Titer controls	0	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	7.875 ± 0.182	
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0.375	Pi = 0	± 0.182
Annulment controls	0	60	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	8.000 ± 0.188	
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0.500	Pi = 0	± 0.188
Test	0.60%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	6.875 ± 0.182	
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0	± 0.182	
Test	0.96%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44444444	7.625 ± 0	
			Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 1.0	Pi = 0.125	Pi = 0	± 0
Test	0.60%	60	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
			Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	Pi = 0	± 0
Test	0.96%	10	xxxxxxx	44444444	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	3.500 ± 0	
			Pi = 0	Pi = 1.0	Pi = 0	Pi = 0	Pi = 0	± 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 Ig dilution									m ± S <sub>m</sub>	
			2	3	4	5	6	7	8	9			
Adenovirus	0	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	00000000	00000000	00000000	6.500 ± 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	44444444 Pi = 1.0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	6.500 ± 0
	0	60	44444444	44444444	44444444	44444444	44444444	44444444	44444444	00000000	00000000	00000000	6.500 ± 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	44444444 Pi = 1.0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	6.500 ± 0
	0.05%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	30000000	00000000	00000000	6.625 ± 0.125
			44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	00000000 Pi = 0.125	00000000 Pi = 0	00000000 Pi = 0	6.625 ± 0.125
	0.10%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	40000000	00000000	00000000	6.625 ± 0.125
			44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	00000000 Pi = 0.125	00000000 Pi = 0	00000000 Pi = 0	6.625 ± 0.125
	0.50%	0	44444444	44444444	22242222	00000000	00000000	00000000	00000000	00000000	00000000	00000000	4.500 ± 0
			44444444 Pi = 1.0	44444444 Pi = 1.0	22242222 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	4.500 ± 0
0.60%	0	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		xxxxxxx	00000000 Pi = 0	00000000 Pi = 0	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	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		xxxxxxx	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	≤2.5 ± 0	
0.60%	60	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	8.000 ± 0.188	
		xxxxxxx	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	8.000 ± 0.188	
0.96%	10	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	8.000 ± 0.188	
		xxxxxxx	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	00000000 Pi = 1.0	8.000 ± 0.188	
Poliovirus	0.60%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44000000	00000000	6.750 ± 0.163	
			44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	00000000 Pi = 0.250	00000000 Pi = 0	00000000 Pi = 0	6.750 ± 0.163
0.96%	0	44444444	44444444	44444444	44444444	44444444	44444444	44444444	44400000	00000000	00000000	6.875 ± 0.182	
		44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	44444444 Pi = 1.0	00000000 Pi = 0.375	00000000 Pi = 0	00000000 Pi = 0	6.875 ± 0.182	
0.60%	60	xxxxxxx	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	00000000	≤2.5 ± 0	
		xxxxxxx	00000000 Pi = 0	00000000 Pi = 0	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	xxxxxxx	44444444	44222200	44222200	00000000	00000000	00000000	00000000	00000000	00000000	4.250 ± 0.163	
		xxxxxxx	44444444 Pi = 1.0	44222200 Pi = 0.750	44222200 Pi = 0.750	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	00000000 Pi = 0	4.250 ± 0.163	