

### Use of an isopropanol-containing solution to combat caliciviridae

The present invention relates to an isopropanol-containing composition and its use in a method of combating Caliciviridae (caliciviruses) on the skin. In addition, the invention relates to the use of this composition to combat Caliciviridae on non-living surfaces.

The combating of viruses plays a crucial role in a large number of technical fields. Caliciviridae, and in particular noroviruses, have become the focus of interest in recent years, in particular in disinfection in hospitals. Disinfection of the hands and of surfaces are in this case essential methods of breaking the infection chain.

EP 0 547 727 A1 describes compositions which can be used as a skin antiseptic and a hand disinfection agent. Effectiveness against viruses is not described. WO 98/44792 A1 discloses the effectiveness of alcohols (in particular ethanol and n-propanol) when mixed with salts at a low temperature against viruses.

EP 2 135 507 A1 discloses a virucidal disinfectant which comprises (a) 40 to 90% by weight ethanol, isopropanol or a mixture thereof, (b) 0.1 to 2% by weight of lactic acid, (c) 0.01 to 2% by weight of citric acid and (d) 0.001 to 0.1% by weight of a zinc containing compound which releases zinc in solution.

EP 1 685 854 A1 discloses a virucidal composition comprising (a) 0.2 to 1.5% by weight of one or several phosphorous containing acid, (b) 30 to 80% by weight, of ethanol, propanol-1, propanol-2 or of a mixture of two or three of them and (c) one or several polyalkylenglycol.

JP 200035506 A disclose a germicidal composition mild to the human body which comprises (A) 30 to 90 Vol.% alcohols such as ethanol, isopropanol or mixture thereof, (B) 0.01 to 5 Vol.% moisturizing agent such as glycerol, triglycerol, ethyleneglycol, a polyethylene glycol sorbitol or a POE methyl glycoside and (C) water in the balance.

By way of example, disinfecting agents based upon ethanol and acting as a virucide are recommended for the hygiene and disinfection of the hands. A virucidal activity has been shown for the product desderman<sup>®</sup> pure of Schülke & Mayr GmbH (78% by weight of ethanol 96%, 0.1% by weight of biphenyl-2-ol). There are nevertheless reservations concerning disinfecting agents for hygienic and surgical hand disinfection which contain large quantities of ethanol, since agents of this type have a strong degreasing effect.

The product desmanol<sup>®</sup> industrial pure of Schülke & Mayr GmbH, on the other hand, contains 32% by weight of propanol-1 (n-propanol) and 21% by weight of

propanol-2 (isopropanol). The product is used undiluted for hand disinfection and is effective against lipophilic viruses. Agents with a content  $\geq 3\%$  n-propanol, however, must be classified as R41 ("risk of serious damage to eyes"), together with the symbol "irritant"; from the 1st of June 2015 preparations containing  $\geq 3\%$  n-propanol must be classified as H318 (GHS classification "causes serious eye damage") and a "corrosive" symbol.

In order to combat viruses in the disinfection of surfaces, the Robert Koch Institute (the German Federal Institute for Infectious Diseases and Non-Communicable Diseases) recommends disinfection agents with a content of per-compounds or aldehydes as active substances (Epidemiological Bulletin 4/2009). In addition, in accordance with *Wallhäußers Praxis der Sterilisation, Desinfektion, Antiseptik und Konservierung* [Wallhäusser's Practice of Sterilization, Disinfection, Antiseptics and Conservation] (Kramer & Assadian, Publ. Thieme Verlag 2008) only substances with an oxidizing action, such as aldehydes, halogens (in particular, active chlorine) and compounds releasing active oxygen (such as peracids, perborates and hydrogen peroxides), are effective against non-enveloped viruses.

The human norovirus causes more than 80% of all types of infectious gastroenteritis. It has been shown in various tests that the effectiveness of isopropanol against noroviruses is limited. In addition, for combating noroviruses on non-living surfaces, Magulski *et al.* (BMC-Infectious Diseases 2009, 9:107) therefore recommend products on the basis of peracetic acid, glutaraldehyde, ethanol or n-propanol. In the tests by Magulski *et al.*, the murine norovirus was used as a surrogate for the human norovirus. In the highest concentration tested of 60% by volume the effectiveness of isopropanol lay three decadic levels below the effectiveness of ethanol and n-propanol. These results are also confirmed by Eggers (International Conference on Prevention and Infection Control, Schülke Satellite Symposia, Geneva 2011).

The subject-matter of the present invention is a disinfectant which is active against Caliciviridae to be used for skin, hand and surface disinfection. The disinfection agent is effective against noroviruses, and more particularly against the murine norovirus. Furthermore, the disinfectant is easy to formulate and has the least possible degreasing effect when used for hygienic and surgical hand disinfection.

That is the reason why, according to a one aspect, there is provided a composition which contains:

- a) - At least 60% by weight and at most 95% by weight of isopropanol, and
- b) - At least 0.2% by weight and at most 4.0% by weight of one or more relipidizing agents chosen from

lauryl alcohol, myristyl alcohol, cetyl alcohol, stearyl alcohol;

c) - 0.05 to 2.0% by weight of at least one or several ingredients chosen from , a combination of sorbitol and despanthenol, or a combination of sorbitol, dexpanthenol and 1-(2-éthyl hexyl) glycerol ether.

5           In another aspect, there is provided a composition as disclosed herein, for use in therapy.

          In a further aspect, there is provided a use of a composition as disclosed herein in the manufacture of a medicament, as a skin disinfectant for treatment of the human or animal body by therapy.

10           In yet another aspect, there is provided a use of a composition as disclosed herein as a disinfectant of non living surface.

          The invention is based *inter alia* upon the fact that it has surprisingly been found

that the effectiveness against Caliciviridae of the composition as hereinabove defined disinfection agents based upon isopropanol and relipidizing agents is similar to the effectiveness of disinfection agents based upon ethanol. This result was not predictable since it had previously been assumed that even in a high concentration isopropanol is not sufficient to destroy Caliciviridae. Moreover, it has additionally been found that relipidizing agents as component b) do not inhibit the effect of isopropanol against Caliciviridae.

In this case the evidence of the effect upon the skin in a practical trial on test persons is decisive. This effect is surprising since, although previous experiments in the suspension test had proved a 4-log level reduction, previous experiments on the skin had shown the opposite. Hitherto, therefore, the effectiveness of isopropanol upon the murine norovirus (MNV) and the feline calicivirus (FCV) had been shown only in tests *in vitro* (suspension tests). The fact that conclusions on the effectiveness upon the skin cannot be drawn directly from the results of suspension tests, however, had already been shown by Eggers (*loc. cit.*).

In the hereinabove defined composition, the content of isopropanol preferably amounts at most to 95% by weight and preferably at most to 90% by weight, preferably from 65% to 85% by weight, in particular from 70% to 80 % by weight, for example from 72% to 78% by weight, such as approximately 75% by weight.

In the hereinabove defined composition, by a relipidizing agent, it is referred to "ein Rückfetter" in German language. The total content of b) relipidizing agents preferably amounts at most to 4.0% by weight, preferably from 0.5 to 2.5% by weight, in particular from 0.8 to 2.0% by weight.

Therefore, according to a particular embodiment, the composition as hereinbefore defined, contains:

- a) - At least 60% by weight and at most to 95% by weight of isopropanol, and
- b) - At least 0.2% by weight and at most 4.0% by weight of one or more relipidizing agents; it particularly contains:

- a) - 60% by weight to 90% by weight of isopropanol, and
- b) - 0.2% by weight to 2.5% by weight of one or more relipidizing agents; and more particularly contains:

- a) - At least 65% by weight and at most to 85% by weight of isopropanol, and
- b) - At least 0.5% by weight and at most 2.5% by weight of one or more relipidizing agents.

According to a very particular embodiment of the composition as hereinbefore defined, contains:

a) - At least 70% by weight and at most to 80% by weight of isopropanol, and  
b) - At least 0.5% by weight and at most 2.5% by weight of one or more relipidizing agents.

By way of example, the relipidizing agents are selected from:

- 5 (i) - (partial) esters of (poly)glycerol, for example glycerol (mono-, di-, tri-)  
[adipate, alkanoate ( $C_8$ ,  $C_{10}$  and  $C_{18}$ ), isostearate], triglycerol diisostearate,  
polyglycerol-2 dipolyhydroxystearate, glycerol oleate, und glycerol cocoate,  
(ii) - lanolin and lecithin as well as polyethoxylated or acylated lanolin and  
lecithin derivatives,  
10 (iii) - polyols such as glycerol, erythritol, 1,2,6-hexanetriol, inositol, lactitol,  
maltitol, mannitol, methylpropanediol, phytantriol, polyglycerols, sorbitol and xylitol,  
wherein glycerol is particularly preferred,  
(iv) - fatty alcohols, *i.e.*, univalent, primary alcohols derived from unbranched,  
optionally mono- or polyunsaturated hydrocarbon residues with from 6 to 22 carbon  
15 atoms, such as lauryl alcohol, myristyl alcohol, cetyl alcohol and stearyl alcohol, and  
(v) - esters such as sorbitan laurate, isopropyl myristate, isopropyl palmitate and  
cetyl stearyl acetate.

According to another particular embodiment, the composition as hereinbefore  
defined, is characterized in that the component b) is glycerol and more particularly in  
20 that it comprises at most 2% by weight of glycerol, preferably at most 1.0% by weight  
and in particular, at most 0.5% by weight of glycerol.

According to another particular embodiment, the composition as hereinbefore  
defined, is characterized in that the component b) is a mixture of at least two or more  
fatty alcohols. According to this embodiment said mixture of two or more fatty alcohols  
25 is a mixture of cetyl alcohol and of stearyl alcohol or a mixture of cetyl alcohol, stearyl  
alcohol and myristyl alcohol.

According to another particular embodiment, the composition as hereinbefore  
defined, is characterized in that it further comprises at least another relipidizing agent  
different from glycerol. According to this embodiment said at least another relipidizing  
30 agent different from glycerol is a mixture of cetyl alcohol, stearyl alcohol and myristyl  
alcohol.

According to another particular embodiment the composition as hereinbefore  
defined further contains:

- c) - From 0.05 to 2.0% by weight of at least one or several further ingredients.  
35 Such ingredients are for example humectants such as sorbitol, and urea and/or  
dexpanthenol as a skin protection and a medicinal skin care agent or agents. In

addition, glycerol ether can be contained in the disinfection agent, as disclosed in EP 0547 727 A, in particular 1-(2-ethylhexyl) glycerinether (Sensiva® SC50).

According to this embodiment, the composition is more particularly characterized in that said at least one several ingredients is chosen from sorbitol, urea, dextranthenol, 1-(2-éthyl hexyl) glycerol ether, a combination of sorbitol and despanthenol, or a combination of sorbitol, dextranthenol and 1-(2-éthyl hexyl) glycerol ether.

According to a more particular embodiment, the composition as hereinabove defined, is characterized in that It consists of, for 100% by weight:

- 10 a) - At least 60% by weight and at most to 95% by weight of isopropanol, and
- b) - At least 0.2% by weight and at most 4.0% by weight of one or more relipidizing agents,
- c) - From 0.05 to 2.0% by weight of at least one or several further ingredients.
- d) - Water as the remainder.

15 In addition, agents used according to the invention are preferably characterized in that they have a slight content of peroxides, such as hydrogen peroxide or peracetic acid, as are frequently used for combating Caliciviridae (and in particular noroviruses). It is preferable for the amount of peroxide to be at most 1.0% by weight, more preferably at most 0.5% by weight, and in particular at most 0.1% by weight, and in a particularly preferred manner the disinfection agent is substantially free of peroxide.

20 In addition, disinfection agents used according to the invention are preferably characterized in that they have a low content of aldehydes (such as glutaraldehyde or formaldehyde), as typically used for combating Caliciviridae (and in particular noroviruses). It is preferable for the amount of aldehyde to be at most 1.0% by weight, preferably at most 0.5% by weight, in particular at most 0.1% by weight, and the disinfection agent is, in particular, preferably substantially free of aldehyde.

30 In addition, agents used according to the invention are preferably characterized in that they have a low content of cationic surfactants, such as quaternary ammonium salts and chlorhexidine derivatives, as frequently used in disinfection agents. It is preferable for the amount of cationic surfactant to be at most 1.0% by weight, preferably at most 0.5% by weight, in particular at most 0.1% by weight, and the disinfection agent is, in particular, preferably substantially free of cationic surfactant.

35 According to another embodiment, the invention relates to the composition as hereinabove defined, for use in a method for treatment of the human or animal body by therapy, and more particularly for use as a skin disinfectant in a method for treatment of the human or animal body by therapy.

Said composition is effective against Caliciviridae (caliciviruses). These are mainly viruses of the genus norovirus, and in particular the human norovirus or the murine norovirus. Alternatively, the Caliciviridae are viruses of the genus vesivirus, and in particular the feline calicivirus.

- 5 Most preferably, the composition as hereinabove defined is for use in the treatment of the human skin, such as the hands,

According to the last embodiment, the invention relates to the use of a composition as hereinabove defined, as a disinfectant of non living surface, for example for area disinfection.

- 10 The disinfection agent used according to the invention thus provides the following advantages:

- good effectiveness against a multiplicity of micro-organisms, in particular against Caliciviridae, such as noroviruses or vesiviruses, at the same time as good compatibility with the skin (even with repeated applications),
- 15 – good effectiveness within a short exposure time, and
- effectiveness against Caliciviridae on living and non-living surfaces.

The advantages of the present invention are more particularly shown in the following examples. Percentages refer to the weight, unless indicated otherwise.

## 20 **Examples**

The following formulations were tested:

|                                    | <b>Formulation A</b> | <b>Formulation B</b> | <b>Formulation C</b> |
|------------------------------------|----------------------|----------------------|----------------------|
| Purified water, %                  | 23.30                | 23.50                | 24.00                |
| Isopropanol, %                     | 75.00                | 75.00                | 74.00                |
| Glycerol, %                        | –                    | –                    | 2.00                 |
| Sorbitol 70%, %                    | 0.50                 | 0.50                 | –                    |
| 1-(2-ethylhexyl) glycerin ether, % | 0.20                 |                      |                      |
| Dexpanthenol, %                    | 0.20                 | 0.20                 | –                    |
| Myristyl alcohol, %                | 0.50                 | 0.50                 | –                    |
| Cetyl stearyl alcohol, %           | 0.30                 | 0.30                 | –                    |
| Appearance                         | clear,<br>colourless | colourless,<br>clear | colourless,<br>clear |

**Method A: Quantitative suspension test of virucidal effect according to EN 14476: Murine norovirus (low stressing)**

Storage conditions: in the original packaging at room temperature

Packaging: plastic bottle

**Methodology:** EN 14476:2005-04/A1:2006-10 and PrEN 14476:2011: Chemical disinfection agents and antiseptics – Quantitative suspension test of virucidal effect for chemical disinfection agents and antiseptics used in human medicine, Test methods and requirements (phase 2, stage 1)

For the demonstration of the virucidal effectiveness according to EN 14476, incubation of suspensions of 8 parts by volume of the disinfection agent (product test solution) with one part of stressing substance (0.3% in the testing of dirty conditions or 0.03% in the testing of clean conditions) and one part by volume of virus suspension is carried out with a titre of at least  $10^7$  TC ID<sub>50</sub> / ml. In order to achieve the concentration of 97%, 0.1 ml of the virus, 0.2 ml of the 5-fold concentrated stressing substance and 9.7 ml of the product are mixed. After the termination of the exposure time, the effect of the disinfection agent is stopped by dilution (or gel filtration) and a series of dilutions in accordance with the progression 1:10 / 1:100 / 1:1,000 *etc.* is applied in order to determine the residual virus content. The titration is carried out in the microtitre system on 96-well plates. In addition, the following controls are carried out: virus control, cytotoxicity control, susceptibility control and reference inactivation with 0.7% formaldehyde. The calculation of the results is carried out in accordance with Spearman and Kärber and is calculated as the difference between the titre of the virus control and the titre of the product test solution whilst including a 95% confidence interval.

### **Test conditions**

|                            |                                      |
|----------------------------|--------------------------------------|
| Exposure time:             | 30 sec, 60 sec, 120 sec              |
| Stressing:                 | 0.3 g BSA/l (low stressing)          |
| Test temperature:          | 20°C ± 0.5°C                         |
| Concentration of the       |                                      |
| Test product (V/V):        | 97% (V/V), 80% (V/V), 50%            |
| Dilution agent:            | water of standardized hardness       |
| Test virus:                | murine norovirus, strain S 99 Berlin |
| Cell line for propagation: | RAW cells ATCC TIB-71                |
| Titration method:          |                                      |

Virus titration on cells as monolayers in 96-well microtitration plates.  
0.5ml of product test solution is diluted with 4.5 ml of ice-cold DMEM + 2% FBS to a

dilution of  $10^{-8}$ . 100  $\mu$ l of each dilution was pipetted into 6 wells of the microtitration plate.

Method of terminating the effect of the product and reducing the cytotoxicity:

- 5 Gel filtration with MicroSpin columns and dilution to  $10^{-4}$  within 10 sec with ice-cold DMEM + 2% FBS

Susceptibility testing:

Comparative virus titrations were carried out on cells which had been treated with 0.1% product solution or PBS.

Reference virus inactivation: 0.7% formaldehyde solution.

- 10 Exposure times: 5, 15, 30, 60 min

### **Calculation of the virucidal effectiveness**

The TCID<sub>50</sub> was calculated in accordance with the method of Spearman and Kärber.

15 
$$m = x_k + d/2 - d \sum p_i$$

$m$  = negative logarithm of the titre in relation to the test volume

$x_k$  = logarithm of the smallest dilution stage in which all the test objects react positively

$d$  = logarithm of the dilution factor

- 20  $p_i$  = observed reaction rate

### **Calculation of the standard deviation**

$$S_m = \sqrt{d^2 \sum [P_i \times (1 - P_i) \div (n - 1)]}$$

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$S_m$  = standard deviation of the logarithmized titre

$d$  = logarithm of the dilution factor

$p_i$  = observed reaction rate

$n$  = number of tests per dilution

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### **Calculation of the reduction factor (RF)**

$$RF = \log (\text{virus control}) - \log (\text{test product})$$

$$K_{RF} = \sqrt{(2S_{m(\text{control})})^2 + (2S_{m(\text{residual titre})})^2}$$

$K_{RF}$  = 95% confidence interval of the reduction factor

$S_m$  = standard deviation of the logarithmized titre

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A summary of the results of the quantitative suspension test in accordance with EN 14476 with the preferred formulation A and murine norovirus is given in Tables 1 and 2. The results of the controls are summarized in Table 3.

#### 10 **Method B:: Modified test EN 1500 “Hygienic disinfection of the hands”**

The test is based upon EN 1500 (practical test method for the hygienic disinfection of the hands).

15 In the tests, the hands of test persons are contaminated with 3 ml of murine norovirus, namely both the hands of the test persons of the reference group and the hands of the test persons of the test group. A sample is then taken (1 min) before the disinfection. After that, the test persons of each group disinfect their hands with disinfection agent. The test persons of the reference group disinfect twice with 3 ml of a reference product in each case, the reference product being allowed to act for 30 sec  
20 and rubbed in after each disinfection. The test persons of the test group instead disinfect twice with 3 ml of the disinfection agent to be tested. After that, a sample is taken for the disinfection (1 min), both in the reference group and in the test group.

25 The test persons of the respective group are then swapped over in a second test.

#### **Example 1**

#### **Results of murine norovirus (method A)**

30 Table 1: Summary of the results of formulation A and murine norovirus

| Product concentration                                                                                                                                           | Stressing   | CD <sub>50</sub> | log <sub>10</sub> -TCID <sub>50</sub> / 0.1 ml after ... sec<br>murine norovirus |               |               | Titre reduction<br>≥ 4 log <sub>10</sub><br>after...sec |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|------------------|----------------------------------------------------------------------------------|---------------|---------------|---------------------------------------------------------|
|                                                                                                                                                                 |             |                  | 30                                                                               | 60            | 120           |                                                         |
| 97% formulation A                                                                                                                                               | 0.3 g BSA/l | ≤ 2·50           | ≤ 2.50 ± 0.00                                                                    | ≤ 2.50 ± 0.00 | ≤ 2.50 ± 0.00 | 30                                                      |
| 80% formulation A                                                                                                                                               | 0.3 g BSA/l | ≤ 2·50           | ≤ 2.50 ± 0.00                                                                    | ≤ 2.50 ± 0.00 | ≤ 2.50 ± 0.00 | 30                                                      |
| 50% formulation A                                                                                                                                               | 0.3 g BSA/l | ≤ 2·50           | 6.00 ± 0.42                                                                      | 5.50 ± 0.00   | 5.83 ± 0.42   | —                                                       |
| Virus control<br>1 ml virus                                                                                                                                     | 0.3 g BSA/l | n.a.             | n.c.                                                                             | n.c.          | 7.50 ± 0.47   | n.a.                                                    |
| Virus control<br>0.1 ml virus                                                                                                                                   | 0.3g BSA/l  | n.a.             | n.c.                                                                             | n.c.          | 6.33 ± 0.54   | n.a.                                                    |
| TCID <sub>50</sub> tissue culture infectious dose<br>CD <sub>50</sub> cytotoxic dose<br>n.a. not applicable<br>n.c. not carried out<br>BSA bovine serum albumin |             |                  |                                                                                  |               |               |                                                         |

#### Results of murine norovirus (method A)

Table 2: Summary of the results of formulation A and murine norovirus

| Product concentration                                                                                            | Stressing   | CD50   | Virus control<br>[log <sub>10</sub> -TCID <sub>50</sub> /<br>0·1 ml]<br>murine norovirus | Reduction factor<br>[log <sub>10</sub> -TCID <sub>50</sub> / 0·1 ml] |                  |                  |
|------------------------------------------------------------------------------------------------------------------|-------------|--------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------|------------------|
|                                                                                                                  |             |        |                                                                                          | 30                                                                   | 60               | 120              |
| 97%<br>formulation A                                                                                             | 0.3 g BSA/l | ≤ 2.50 | 6.33 ± 0.54                                                                              | ≥ 3.83 ±<br>0.54                                                     | ≥ 3.83 ±<br>0.54 | ≥ 3.83<br>± 0.54 |
| 80%<br>formulation A                                                                                             | 0.3 g BSA/l | ≤ 2.50 | 7.50 ± 0.47                                                                              | ≥ 5.00 ±<br>0.47                                                     | ≥ 5.00 ±<br>0.47 | ≥ 5.00<br>± 0.47 |
| 50%<br>formulation A                                                                                             | 0.3 g BSA/l | ≤ 2.50 |                                                                                          | 1.50 ±<br>0.63                                                       | 2.00 ±<br>0.47   | 1.67 ±<br>0.63   |
| TCID <sub>50</sub> tissue culture infectious dose<br>CD <sub>50</sub> cytotoxic dose<br>BSA bovine serum albumin |             |        |                                                                                          |                                                                      |                  |                  |

5 **Results of murine norovirus (method A)**

Table 3: Virus controls and reference inactivation with murine norovirus

| Product concentration                                                                                                                                                                                             | Stressing   | CD <sub>50</sub> | log <sub>10</sub> -TCID <sub>50</sub> / 0.1 ml after ... min murine norovirus |             |             |             |             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|------------------|-------------------------------------------------------------------------------|-------------|-------------|-------------|-------------|
|                                                                                                                                                                                                                   |             |                  | 0                                                                             | 5           | 15          | 30          | 60          |
| Formaldehyde 0.7% (V/V)                                                                                                                                                                                           | PBS         | ≤ 3.5            | n.c.                                                                          | 6.33 ± 0.33 | 5.67 ± 0.54 | 5.17 ± 0.56 | 4.33 ± 0.33 |
| Virus control 20°C 1 ml virus                                                                                                                                                                                     | 0.3 g BSA/l | n.a.             | 7.50 ± 0.47                                                                   | n.c.        | n.c.        | n.c.        | n.c.        |
| Virus control 20°C 0.1 ml virus                                                                                                                                                                                   | 0.3 g BSA/l | n.a.             | 6.33 ± 0.54                                                                   | n.c.        | n.c.        | n.c.        | n.c.        |
| Susceptibility (PBS)                                                                                                                                                                                              | 0.3 g BSA/l | n.a.             | n.c.                                                                          | n.c.        | n.c.        | n.c.        | 7.67 ± 0.62 |
| Susceptibility (0.1% formulation A)                                                                                                                                                                               | 0.3 g BSA/l | n.a.             | n.c.                                                                          | n.c.        | n.c.        | n.c.        | 7.67 ± 0.33 |
| n.a. not applicable<br>n.c. not carried out<br>WSH water of standardized hardness<br>PBS phosphate-buffered salt solution<br>TCID <sub>50</sub> tissue culture infectious dose<br>CD <sub>50</sub> cytotoxic dose |             |                  |                                                                               |             |             |             |             |

**Verification of the method**

- Cytotoxicity:** After gel filtration, the 97%, 80% and 50% products of formulation A with a dilution of  $10^{-2}$  (2.5 CD<sub>50</sub>) displayed no cytotoxic effect. This consequently does not affect the necessary dilutions to prove a virucidal effect.
- Virus control:** The virus titre for the murine norovirus amounted to  $7.50 \pm 0.47$  TCID<sub>50</sub> / 0.1 ml and thus to  $8.50 \pm 0.47$  TCID<sub>50</sub> / ml. The demonstrable titre reduction thus amounted to  $\geq 4.00 \log^{10}$ .
- Susceptibility:** The comparative virus titration on pre-treated and untreated cells displayed a difference of  $< 1.00 \log^{10}$  levels.
- Reference inactivation** The reduction factor of 0.7% formaldehyde amounted to  $2.33 \log^{10}$  after 30 min exposure time and to  $3.17 \log^{10}$  after 60 min exposure time.

## **Results**

- 5 After an exposure time of 30 sec with the 97% formulation A with a low stressing, no propagation of murine norovirus could be shown in cultures of RAW cells. It was already possible to demonstrate the necessary titre reduction of 4 log<sub>10</sub> levels for an 80% application concentration.

### **Example 2 (method B)**

|                                    | <b>Formulation B</b> | <b>Formulation C</b> | <b>Ethanol 70% w/w</b> |
|------------------------------------|----------------------|----------------------|------------------------|
| <b>log TCID<sub>50</sub>/ml</b>    | <b>2 × 30 s</b>      | <b>2 × 30 s</b>      | <b>2 × 30 s</b>        |
| <b>Average before disinfection</b> | <b>563</b>           | <b>5.28</b>          | <b>5.6</b>             |
| Standard deviation                 | 0.44                 | 0.38                 | 0.39                   |
| <b>Average after disinfection</b>  | <b>2.75</b>          | <b>2.58</b>          | <b>3.02</b>            |
| Standard deviation                 | 0.08                 | 0.23                 | 0.65                   |
| <b>RF</b>                          | <b>2.88</b>          | <b>2.70</b>          | <b>2.58</b>            |
| Standard deviation                 | 0.45                 | 0.43                 | 0.73                   |
| <b>Number of test persons</b>      | <b>5</b>             | <b>5</b>             | <b>5</b>               |

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- The above data show that the combinations of isopropanol (75% by weight) with relipidizing agents (formulation B), according to the invention, have a better effect than 70% ethanol. In addition, it was surprising that the effect of a disinfection agent according to the invention with the combination of relipidizing agents (B) is even better
- 15 than in the presence of glycerol as the sole relipidizing agent (formulation C).

### Claims

1. A composition which contains:

a) - At least 60% by weight and at most to 95% by weight of isopropanol, and

5        b) - At least 0.2% by weight and at most 4.0% by weight of one or more  
relipidizing agents chosen from lauryl alcohol, myristyl alcohol, cetyl alcohol, stearyl  
alcohol;

10        c) - 0.05 to 2.0% by weight of at least one or several ingredients chosen from a  
combination of sorbitol and despanthenol, or a combination of sorbitol, dexpantenol  
and 1-(2-éthyl hexyl) glycerol ether.

2. A composition according to Claim 1, characterized in that component b) is a  
15        mixture of cetyl alcohol and of stearyl alcohol.

3. A composition according to Claim 1, characterized in that

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the component b) is a mixture of cetyl alcohol, stearyl alcohol and myristyl alcohol.

4. A composition according to anyone of Claims 1 to 3, for use in therapy.

5            5. Use of a composition according to anyone of Claims 1 to 3 in the manufacture of a medicament, as a skin disinfectant for treatment of the human body.

6. Use of a composition according to anyone of Claims 1 to 3, as a disinfectant of non living surface.