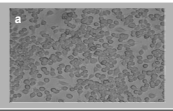
Effectiveness of alcohols, hand rubs and scrubs against murine norovirus – a surrogate for human norovirus

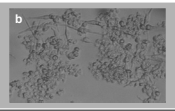
Jochen Steinmann[1], Britta Becker[1], Birte Bischoff[1], Dajana Paulmann[1], Jörg Steinmann[2] and Eike Steinmann[3]

[1] MikroLab GmbH, 28259 Bremen, Germany
[2]Institute of Medical Microbiology, University Hospital, 45147 Essen, Germany
[3]Division of Experimental Virology, TWINCORE, Centre for Experimental and Clinical Infection Research, 30625 Hannover, Germany

Abstract

Background/Objectives: Human noroviruses (HNV) are a significant cause of non-bacterial gastroenteritis worldwide. HNV contribute to a lot of outbreaks in hospitals and other public or medical settings. In the past, the cultivable feline calicivirus (FCV) known as a respiratory pathogen has been used as a surrogate for HNV. Recently, the propagation of the murine norovirus (MNV) in dendritic cells and macrophages was achieved. This virus is typically spread by a fecal-oral route and is biologically very similar to human NoV. In the genus norovirus MNV is the only member that replicates in cell culture and in small animals. It is assumed that viruses that use the same transmission route possess comparable stabilities.
Methods: In this study, we wanted to address the stability of MNV towards chemical disinfectants. The isolate S99 of the Robert Koch-Institute Berlin was used as test virus. Hand rubs, hand scrubs and different kinds of alcohol as active ingredients were tested in a quantitative suspension test according to EN 14476:2007-02. Additionally, fingerpads experiments following ASTM 1838-02 were incorporated to study the *in vivo* inactivation of MNV.
Results: Results of the quantitative suspension tests revealed that hand rubs based upon high concentrations of ethanol (> 80.0% v/v) were superior to those based upon 2-propanol or a mixture of 2- and 1-propanol. Even 15 seconds were sufficient to achieve a four $\log_{10}$-reduction of virus titer by these biocides in suspension tests. Ethanol as active ingredient was also found to be more effective than both 1-propanol and 2-propanol. The 60 % (v/v) concentrations were able to reach the following reduction factors (RF) after 60 seconds exposure time: > 6.44 (ethanol), 5.43 (1-propanol) and 2.73 (2-propanol). Two scrubs were not able to reduce MNV after five minutes contact time (RF < 1.0). A third product based upon PVP Iodine achieved a 2.19 $\log_{10}$-reduction within five minutes. Finally, fingerpad experiments demonstrated that ethanol reached also higher RF than 2-propanol after 30 seconds.
Conclusions: Our results clearly demonstrate that ethanol based hand rubs are in favour for inactivation of MNV. *In vitro* suspension tests may be a valuable predictor of *in vivo* activity as measured by the fingerpads method. Based upon our data with the different kinds of alcohol, MNV is regarded as a hydrophilic virus with some lipophilic character.





a RAW cells mock infected
b MNV infected RAW cells

Methods

Biocides: The different concentrations of alcohols (Sigma-Aldrich, Germany) were prepared immediately before the inactivation experiments with water of standardized hardness according to EN 14476:2007-02. Hand rubs and scrubs have been used undiluted.
Suspension test according to EN 14476[1]: 0.8 ml of the hand rub, hand scrub, the different kinds of alcohol or standard hard water as control were mixed with 0.1 ml of test virus suspension (MNV; isolate S99, kindly provided by the Robert Koch-Institute in Berlin) and 0.1 ml of the interfering substance (PBS). At the end of the exposure time, an aliquot of the mixture was immediately diluted.
Fingerpads experiment according to ASTM 1838[2]: 10 µl of the virus suspension were pipetted onto a demarcated area on fingerpads of human volunteers. After air drying the areas were exposed to the biocides or the water control for 30 sec. After that, remaining virus was eluted with EBSS and titrated onto permissive RAW cells.
Virus titration and determination of infectivity:
100 µl of each dilution from inactivation experiments were placed in eight wells of a sterile 96-well microtiter plate with the permissive RAW 264.7 (ATCC TIB-71).
Plates were incubated for 5 days and infectivity was analyzed by virus-induced cytopathic effect. Virus titers were determined by the method of Spearman[3] and Kärber[4]. Titer reduction was calculated by subtracting the virus titer ($TCID_{50}$/ml) of the biocide from the virus titer of the appropriate control.

Effectiveness of biocides in the suspension test

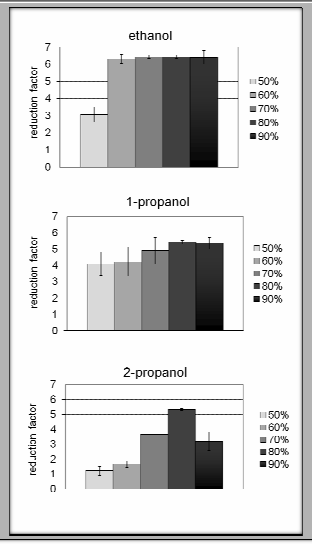


Figure 1: Effectiveness of ethanol, 1- and 2-propanol after 0.5 min of exposure time. Concentrations of alcohols were ranging from 50 to 90 % (v/v).

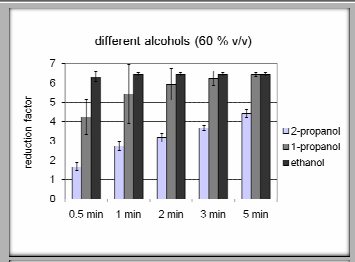


Figure 2: Effectiveness of the alcohols as 60 % (v/v) solutions.

Table 1: ≥ 4 $\log_{10}$ reduction of commercial hand rubs after indicated exposure time.

active ingredients	exposure time
95 g ethanol	15 s
90 g ethanol	15 s
75 g ethanol	15 s
53 g ethanol 20 g 1-propanol 3 g 2-propanol	30 s
57.6 g ethanol 10 g 1-propanol	30 s
45 g ethanol 18 g 1-propanol	30 s
55 g 1-propanol 16 g ethanol	30 s
26 g 1-propanol 47 g 2-propanol	60 s
62 g 2-propanol	300 s

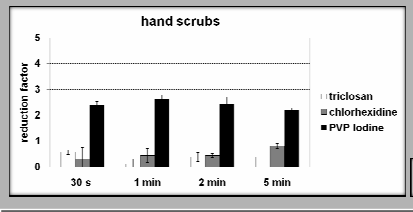


Figure 3: Effect of hand scrubs with different ingredients.

Effectiveness of biocides on fingerpads

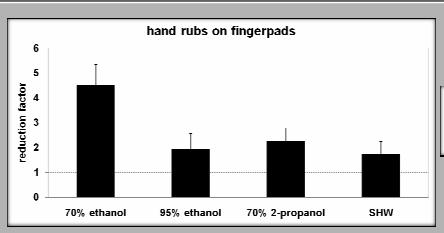


Figure 4: Effectiveness of ethanol (70 % and 95 % v/v) and 2-propanol (70 % v/v) in comparison to standard hard water (SHW) after 0.5 min exposure time.

Results

Results from the suspension tests:
Different alcohols as well as hand rubs and scrubs were evaluated in the quantitative suspension tests within 0.5 min to 5 min of exposure time.

Ethanol was found to be more effective than 1-propanol and 2-propanol, except for the concentration of 50 % (v/v), where 1-propanol reached a higher reduction. 60.0 % (v/v) ethanol was able to reduce the MNV titer after 30 sec by > 6 $\log_{10}$-steps, whereas RF of 4.24 and 1.66 were measured with 1-propanol and 2-propanol. In general, by increasing the content of alcohol or exposure time the reduction factor was also enhanced (see Fig. 1 and 2).
When comparing commercially available hand rubs and scrubs, a 4 $\log_{10}$-reduction of MNV titers after short contact times (15 to 30 sec) could be observed with all hand rubs with more than 60 % (v/v) ethanol and/or 1-propanol as main ingredients (see Tab. 1). In contrast, the PVP Iodine-based hand scrub achieved a maximum reduction of 2.63 $\log_{10}$ after 5 min, whereas the two other hand scrubs with triclosan or chlorhexidine were ineffective against MNV (RF < 1, see Fig. 3).

Results with the fingerpad method:
The *in vivo* experiments with MNV demonstrated that ethanol (70 % v/v) was more effective than 2-propanol (70 % v/v). In contrast to the suspension test a higher concentration of ethanol (95 % v/v) was not able to inactivate MNV in a significant manner. Neither 70 % 2-propanol nor 95 % (v/v) ethanol had the ability to reduce MNV titer more than standard hard water (SHW).

Conclusions

Hand hygiene is one of the most important measures to prevent nosocomial infections caused by viruses requiring a biocide with a proven effectiveness against model viruses and their surrogates.
Our *in vitro* experiments clearly demonstrate that ethanol and 1-propanol are in favour for inactivation of MNV. In addition, higher concentrations of the analysed alcohols were more effective. In contrast, the results of the *in vivo* tests revealed that increasing of alcohol concentration did not enhance effectiveness.
Our results show, that *in vitro* suspension tests may be a valuable predictor of *in vivo* activity, but for use recommendations it is necessary to evaluate effectiveness by *in vivo* methods.

References

1. EN 14476:2007-02. Chemical disinfectants and antiseptics. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (phase 2, step 1). 2007.
2. ASTM E 1838-02. Standard test method for determining the virus-eliminating effectiveness of liquid hygienic handwash and handrub agents using the fingerpads of adult volunteers. ASTM International, West Conshohocken, PA, USA 2002.
3. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242.
4. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487