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Letter to the Editor

Virucidal activity of different alcohols against murine norovirus, a surrogate of human norovirus

Madam,

Human noroviruses (HuNoV) cause many outbreaks of non-bacterial gastroenteritis in hospitals and other public or medical settings. To date, HuNoV has not been cultivated in cell culture. However, propagation of the murine norovirus (MNV) was achieved recently in dendritic cells and macrophages.[1]

Hands play an important role in person-to-person spread of HuNoV. Adequate hand hygiene is therefore one of the most important measures in the prevention of HuNoV transmission. Infection prevention and control guidelines emphasize the importance of hand hygiene procedures using alcohol-based hand rubs.[2,3]

Hand rubs in Europe are mainly based on ethanol and 2-propanol, and 1-propanol is a component in some products. At present, no basic data on the efficacy of hand rubs against norovirus are available. Only limited knowledge exists on the activity of ethanol-based products.[4] Therefore, the aim of this study was to examine the efficacy of active ingredients of alcohol-based hand rubs, followed by a test simulating practical conditions with ethanol and 2-propanol.

In Europe, hand rubs are tested against viruses with a suspension assay in accordance with EN 14476 with poliovirus type 1 and adenovirus type 5.[5] In our study, both viruses were replaced by MNV (isolate S99 of the Robert Koch-Institute, Berlin, Germany) as a surrogate of HuNoV. In contrast to MNV-1, isolate S99 can be used for all scientific purposes.[1]

Various test concentrations of ethanol, 1-propanol and 2-propanol (50–90% v/v) were prepared immediately before the inactivation experiments with water of standardized hardness according to EN 14476.[5] At the end of the exposure time, aliquots of the test mixture were immediately diluted and transferred to *RAW 264.7* cells to determine the virus titre ($TCID_{50}$/mL). The titre reduction [reduction factor (RF)] was calculated by subtracting the virus titre of the test substance from the virus titre of the water control. A 4 $\log_{10}$ reduction (≥99.99%) is necessary to demonstrate virucidal activity.[4]

The effectiveness of ethanol and 2-propanol at eliminating virus under practical conditions was determined by experiments with the fingerpads of four volunteers contaminated artificially with MNV. Tests were performed according to the standard test method of the American Society for Testing and Materials (ASTM Standard E 1838-02).[6] This study was approved by the Ethics Committee of Zentralkrankenhaus St.-Jürgen-Str. (now ZKH Bremen-Mitte) in Bremen, Germany. Briefly, the fingerpads were contaminated with 10 μL MNV test virus suspension and were allowed to become visibly dry. The dried inoculum was then exposed to 1 mL test alcohol in a plastic vial, which was shaken for 30 s with approximately 30 inversions.

To recover residual virus, a plastic vial containing 1 mL Earle's balanced salt solution was placed on the treated area and shaken for 20 s with approximately 20 inversions. An aliquot of this eluent was diluted immediately to determine the virus titre. The level of infectious virus remaining after the inoculum was dried was used as the baseline value to determine the extent of virus elimination.

Results of the quantitative suspension tests with 30 s of exposure, depicted in **Figure 1**(a), revealed that ethanol concentrations of 60–90% (v/v) were superior to 1-propanol and 2-propanol. Comparing the 60% (v/v) concentrations, RFs were 6.32, 4.24 and 1.66 for ethanol, 1-propanol and 2-propanol, respectively. Only a 50% (v/v) concentration of 1-propanol achieved a higher RF than 50% (v/v) ethanol. For 2-propanol, a concentration of 80% (v/v) was required to achieve a 4 $\log_{10}$ reduction after 30 s of exposure. Surprisingly, the 90% (v/v) concentration of 2-propanol was less active against MNV than the 80% (v/v) concentration. In summary, the results clearly demonstrate the superiority of ethanol.

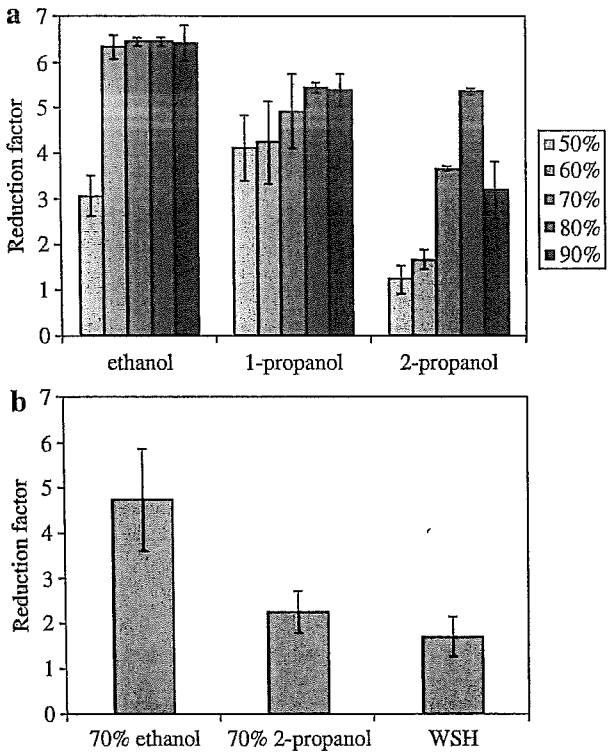

Figure 1. (a) Murine norovirus inactivation by ethanol, 1-propanol and 2-propanol after 30 s of exposure in the suspension test in duplicate. Alcohol concentrations in the test mixture ranged from 50% to 90% (v/v). (b) Effectiveness of 70% ethanol (v/v) and 70% 2-propanol (v/v) on the fingerpads of four volunteers (48 determinations) compared with water of standardized hardness (WSH) after 30 s of exposure.

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Data from the suspension test were confirmed by the fingerpad method. Ethanol achieved a 4.69 $\log_{10}$ reduction in MNV titre, whereas 2-propanol only reached an RF of 2.24 and water of standardized hardness (control for the mechanical effect) only reached an RF of 1.70 [Figure 1(b)].

The use of alcohol-based hand rubs is an important step in the prevention of HuNoV transmission in hospitals and other settings. Our data clearly demonstrate the superiority of ethanol for inactivation of MNV in test concentrations ≥60% (v/v). Results with two World Health Organization formulations based on 80% (v/v) ethanol and 75% (v/v) 2-propanol showed similar effects. The formulation with ethanol was able to achieve a 4 $\log_{10}$ reduction within 30 s in the suspension test, whereas the 2-propanol formulation failed.[7] Furthermore, Belliot *et al.* found that ethanol was active within 30 s (>4 $\log_{10}$), whereas 2-propanol achieved an RF of 3.86.[4]

In conclusion, our study demonstrated the efficacy of ethanol *in vitro* and *in vivo* against MNV as a surrogate of HuNoV. We recommend the use of ethanol-based formulations, with proven efficacy against MNV, in settings with frequent infections and in outbreak situations.

Conflict of interest statement
None declared.

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