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Virucidal activity of a new hand disinfectant with reduced ethanol content: comparison with other alcohol-based formulations

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Summary A new formula with reduced ethanol content (55%) in combination with 10% propan-1-ol, 5.9% propan-1.2-diol, 5.7% butan-1.3-diol and 0.7% phosphoric acid exhibited a broad spectrum of virucidal activity. In quantitative suspension tests, with and without protein load, this formulation reduced the infectivity titre of seven enveloped (influenza A and B, herpes simplex 1 and 2, bovine corona, respiratory syncytial, vaccinia, hepatitis B, bovine viral diarrhoea) and four non-enveloped (hepatitis A, polio, rota, feline calici) viruses $>10^3$-fold within 30 s. In comparative testing, only 95% ethanol showed similar levels of activity.

In fingerpad tests, the formulation produced a $\log_{10}$ reduction factor of the titre of poliovirus type 1 (Sabin) of 3.04 in 30 s compared with 1.32 by 60% propan-2-ol. Testing against feline calicivirus produced a $\log_{10}$ reduction factor of 2.38 by the test formulation; in contrast, the $\log_{10}$ reduction factors with 70% ethanol and 70% propan-1-ol were 0.68 and 0.70, respectively.

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Introduction

Many pathogenic viruses can remain viable on human hands for hours.[1,2] This gives hands the potential to spread such infectious agents directly or indirectly[3-5] in settings such as hospitals.[6,7] Recent studies with experimentally contaminated fingertips have further substantiated the potential of hands to spread viruses.[8,9] These observations re-emphasize the need for proper hand disinfection in the prevention and control of nosocomial outbreaks of viral infections in particular. However, hand disinfectants are often tested against vegetative bacteria only and this may not reflect on their ability to deal with viruses.[10]

While alcohol-based hand rubs generally have a broader and relatively rapid antimicrobial activity, they are often limited in their ability to inactivate non-enveloped viruses.[7] Raising the ethanol content may address this issue to some degree, but increases the risk of tissue toxicity[11] and lowers the flash point. At present, only one formulation with broad virucidal activity exists with an ethanol content of 95 vol%. Therefore, efforts were made to reduce the ethanol content without reducing the virucidal activity to decrease the flash point and increase skin tolerance and compliance. As a result of these efforts, a synergistic combination was developed with an ethanol content of 55% (w/w) in combination with 10% (w/w) propan-1-ol, 5.9% (w/w) propan-1.2-diol, 5.7% (w/w) butan-1.3-diol and 0.7% phosphoric acid.[12] This ready-to-use formulation is registered by the US Food and Drug Administration (NDC-6673-1230-(I)-(9)). Since introduction of the evaluated product in Austrian hospitals, no relevant unwanted side-effects have been reported to date.

Materials and methods

Cells

The following cells were used: FL (amnion) cells (Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria; ATCC No. CCL-62) in Dulbecco's modified Eagle medium (DMEM) (GIBCO BRL, Paisley, Scotland, UK) containing 10% heat-inactivated fetal bovine serum (GIBCO BRL, Grand Island, NY, USA) supplemented with 10 mmol/L HEPES buffer (VWR International GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/mL, streptomycin, 100 μg/mL); BS-C-1 (Cercopithecus monkey kidney) cells (ATCC No. CCL-26, USA) in DMEM; Madin–Darby canine kidney cells (ATCC No. CCL-34) in DMEM; Hep-2 cells (No. NBIMCC-95, National Bank for Industrial Micro-organisms and Cell Cultures, Sofia, Bulgaria) in DMEM; MRC-5 (human embryo lung diploid) cells (ATCC No. X55) in DMEM; Madin–Darby bovine kidney (MDBK) cells (No. NBIMCC-1031) in DMEM; human diploid foreskin fibroblasts (National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria) in DMEM; KE-R cells (provided by Dr Riebe, Cell Bank for Cell Lines in Veterinary Medicine, Federal Research Institute for Animal Virus Diseases, Isle of Riems, Germany) in Eagle's minimum essential medium (EMEM) (Cambrex Bio Science Verviers s.p.r.l., Verviers, Belgium) containing 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany); GMK-AH 1 (Institute of Medical Microbiology, University of Kiel, Germany) in DMEM; Vero cells (ATCC No. CCL81) in DMEM; HRT-18 (human rectal tumour) cells (provided by Dr Herbst, Institute for Animal Hygiene and Infectious Diseases, University of Giessen, Germany) in DMEM; calf trachea cells in DMEM; HepG2 cells (supplied by ATCC cell HB 8065) in DMEM; and MA-104 in EMEM.

Viruses

The virus test strains and their respective culture media were as follows: poliovirus type 1 (Mahoney/Pette, Stephan Angeloff Institute of Microbiology), cultivated in FL cells (maintenance medium DMEM without serum), virus titre 1.3×10^9 plaque-forming units/mL; human rotavirus strain Wa, cultivated in Ma-104 cells without serum, virus titre $10^{7.8}$ cell culture infective dose $CCID_{50}$/mL; hepatitis A virus (HAV, HM 175/18 f cell culture adapted cytopathic clone B, ATCC No. VR-1402), cultivated in BS-C-1 cells (maintenance medium DMEM plus 2% FCS), virus titre $10^{6.8}$ $CCID_{50}$/mL; bovine viral diarrhoea virus (BVDV) (Istituto Zooprofilatice, Peruggia, Italy), cultivated in calf trachea cells (maintenance medium DMEM plus 0.5% FCS), virus titre $10^{7.0}$ $CCID_{50}$/mL; influenza A virus [Aichi/2/68 (H3N2), Stephan Angeloff Institute of Microbiology], cultivated in allantoic fluid of 10-day-embryonated eggs at 37 °C, virus titre $10^{7.5}$ $CCID_{50}$/mL; influenza B virus (Lee/40, ATCC No. VR-101), inoculated in the same manner and cultivated at 35 °C, virus titre $10^{7.5}$ $CCID_{50}$/mL; human rhinovirus (HRV) type 14 strain 1059, ATCC No. VR-284, cultivated in MRC-5 cells (maintenance medium DMEM plus 2% FCS), virus titre $10^{6.6}$ $CCID_{50}$/mL; bovine corona virus (BCV) strain L9 (BCV-L9, provided by Dr Herbst), cultivated in HRT-18, virus titre $10^{7.5}$ $CCID_{50}$/mL; respiratory syncytial virus (RSV) (District Centre of