TWINCORE

Novel Approach for the Validation of Disinfectants against Hepatitis C Virus

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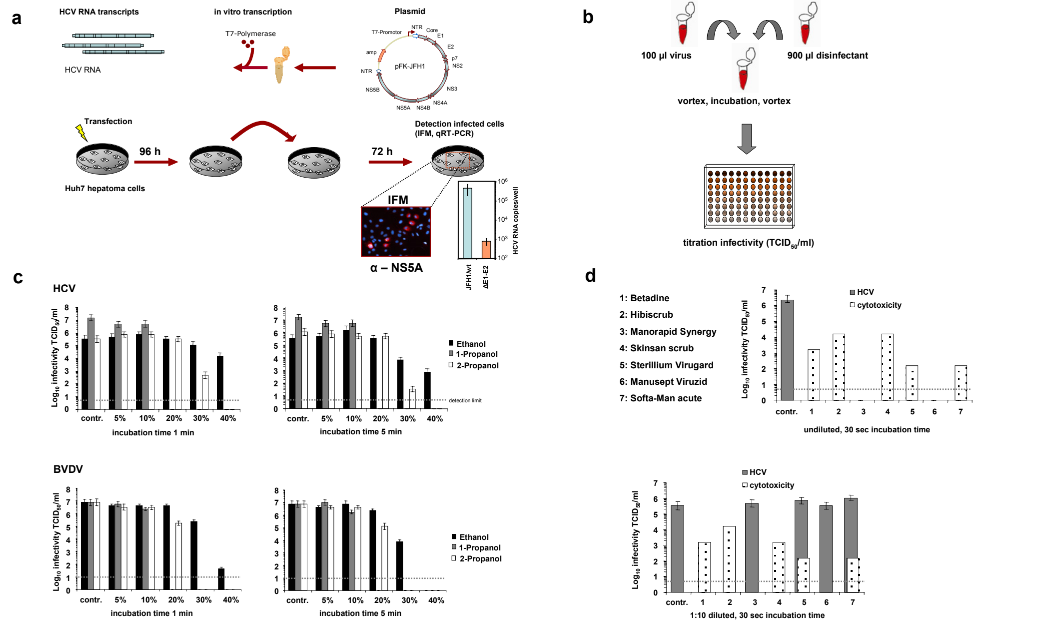
Background and objectives: In the absence of a cell culture system for propagation of the hepatitis C virus (HCV), the antiviral activity of disinfectants against HCV was extrapolated from studies with the bovine viral diarrhea virus (BVDV), an animal pathogen from the same virus family. The recent development of a HCV infection system allowed for the first time direct assessment of environmental stability and susceptibility to chemical disinfectants of this important human pathogen causing nosocomial infections.

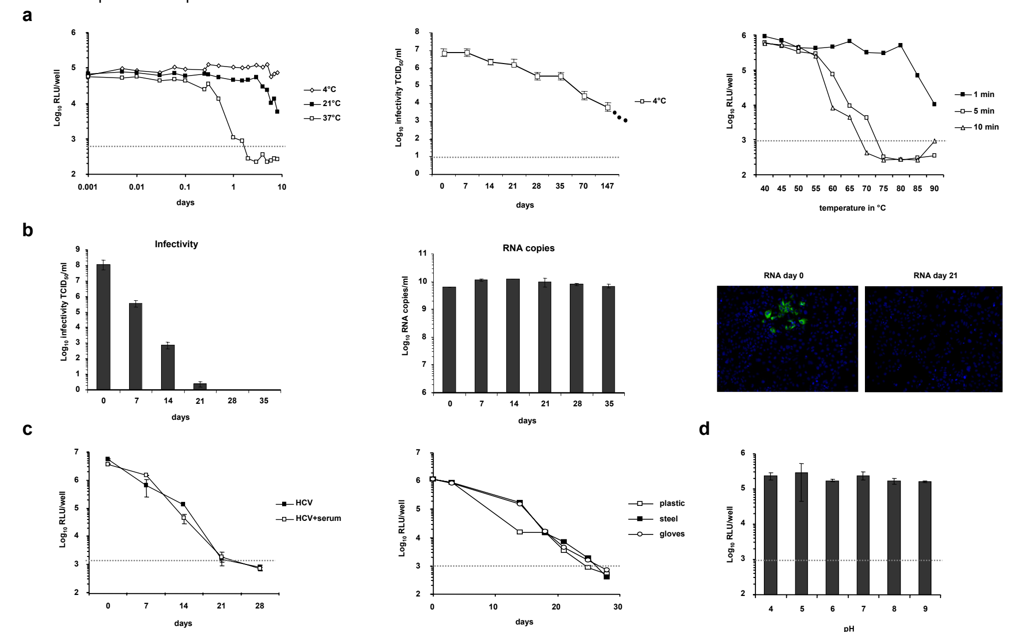
Methods: Inactivation studies were performed in a quantitative suspension assay using cell culture grown HCV without soil load at different exposure times. Residual infectivity was determined by end point dilution method. Thermo stability of HCV was analyzed by incubation of the virus at different temperatures for several days or weeks. HCV RNA levels were analyzed by quantitative real-time PCR. Genome stability was determined by transfection of recovered RNA into highly permissive Huh7.5 cells and immunostaining of NS5A.

Results: The alcohols 1-propanol, 2-propanol and ethanol reduced the infectivity of HCV to undetectable levels within 5 minutes at concentration of 30%, 40% and 50%, respectively. When comparing seven commercially available hand scrubs and rubs with 30 s exposure times, a reduction of virus titers by 6 $\log_{10}$-steps was observed in all samples tested. However, diluting the hand disinfectants to a concentration of 10% abrogated the virucidal activity of the alcohol-based rubs in comparison to the hand wash products. To determine the environmental stability of HCV, we assessed viral infectivity and RNA copy numbers by storing HCV at room temperature for several weeks. Each week, viral titers decreased by 2-3 $\log_{10}$ and after 28 days viral infectivity was completely lost. Analysis of RNA copy numbers revealed no drop in genome copy numbers during this time period.

Conclusions: This study describes a novel system for the validation of chemical disinfectants active against HCV by analyzing virucidal activity of various alcohols and hand disinfectants using a quantitative suspension assay. In addition, HCV stability was determined at different temperatures and data demonstrated no correlation between infectivity and RNA levels. The described assay and these data should be useful to define rigorous disinfection protocols to prevent nosocomial transmission of HCV.



(a) Generation of infectious HCV in cell culture. Based on a DNA plasmid encoding the JFH1 isolate HCV RNA can be generated by in vitro transcription. Huh7 cells were transfected with JFH1 RNA and four days later cell culture supernatant was harvested and used to infect naive Huh7 cells. Infection events can be visualized by NS5A-specific immunofluorescence or quantitative real-time PCR. **(b) Quantitative suspension test.** Viral suspension assay is based on incubation of one part of virus with nine parts of disinfectant reagent. After different exposure times rest-infectivity was determined by a limiting dilution assay based on the detection of NS5A. Viral titers are displayed as tissue culture infectious dose 50 (TCID50). **(c) Effect of ethanol, 1-propanol and 2-propanol on HCV/BVDV.** Ethanol, 1-Propanol and 2-Propanol were tested for their efficacy to inactive HCV (upper panel) and BVDV (lower panel). Disinfectant concentrations were ranging from 5-40% and 1 or 5 min incubation times were used. **(d) Effect of commerical disinfectants against HCV.** Seven different commerical disinfectants were tested for the efficacy to inactive HCV. Incubation times of 30 sec were conducted and different products were used at 80% concentrations (upper panel) or 1:10 diluted (lower panel).



(a) Stability of HCV at different temperatures. HCV was incubated at indicated temperatures and time intervalls. Infectivity was determined by measuring HCV reporter activity (left and right panel, respectively) or by a limiting dilution assay (middle panel). **(b) Correlation of viral infectivity with RNA copy numbers.** We assessed viral infectivity and RNA copy numbers by storing HCV at room temperature for several weeks. RNA copy numbers of respective samples were determined by quantitative RT-PCR. Recovered RNA was re-electroporated into Huh7.5 and HCV positive cells were visualized by immunofluorescence against NS5A. Nuclear DNA was counterstained with DAPI. **(c) Effect of serum, different surfaces and pH on HCV stability.** HCV was incubated at room temperature for several days in the presence and absence of serum and infectivity was determined by HCV reporter activity (left panel). Incubation of virus was conducted on different surfaces (plastic, steel and gloves) before measuring viral reporter activity (middle panel). Importance of pH for viral stability was addressed by incubation of HCV at different pH values following determination of HCV reporter activity.

Conclusions:

- Establishment of an HCV infection system for the experimental analysis of virus stability
- Alcohol as active substances of hand disinfectants inactivate HCV comparable to BVDV
- Dilutions of alcoholic disinfectants do not inactivate HCV
- HCV is quite stable at room temperature and 4°C
- Detection/stability of HCV RNA does not correlate with viral infectivity
- Serum and different surfaces do not alter HCV stability