Übertragung und Inaktivierung des Hepatitis C Virus

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*joint venture between Medical School Hannover and Helmholtz Centre for Infection Research
Stability of human pathogenic viruses

- **With envelope**
  - Strong lipophilic character
  - Weak lipophilic character

- **Without envelope**
  - Hydrophilic character
  - Lipophilic character

**Virus Families**
- **Retroviridae**
- **Flaviviridae**
- **Togaviridae**
- **Herpesviridae**
- **Orthomyxoviridae**
- **Poxviridae**
- **Hepadnaviridae**
- **Picornaviridae**
- **Parvoviridae**
- **Adenoviridae**
- **Reoviridae**
- **Caliciviridae**

Based on Klein and Deforest (1963)
**Family:** Flaviviridae  
**Genus:** Hepacivirus  
**Species:** Hepatitis C virus (7 genotypes)  
**Size:** 50-60 nm  
**Genome:** (+) ssRNA, ~9.6 kb  
**Prevalence:** 160 million patients  
**Therapy:** next talk
Ways of HCV Transmissions

- i.v. drug abuse: 60%
- sex: 15%
- transfusions: 10%
- unknown: 10%
- other: 5%
<table>
<thead>
<tr>
<th>testvirus</th>
<th>surrogate-virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B Virus</td>
<td>Duck hepatitis B virus (DHBV)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>feline Calicivirus (FCV)</td>
</tr>
<tr>
<td></td>
<td>murine Norovirus (MNV)</td>
</tr>
<tr>
<td>Hepatitis C Virus</td>
<td>bovine viral diarrhea virus (BVDV)</td>
</tr>
</tbody>
</table>
**HCV infection system**

- **HCV RNA transcripts**
- **In vitro Transcription**
- **Plasmid**

- **Huh7 hepatoma cells**
  - Transfection
  - 96 h
  - 72 h

- Detection infected cells (IFM, qRT-PCR)

- **HCV RNA copies/well**
  - JFH1/wt
  - ΔE1-E2

- **HCV infection system**
  - Wakita and Pietschmann et al., Nat Med 2005
Properties of cell culture-grown HCV particles

- Density peaks in sucrose gradient ca. 1.15 g/ml
- Spherical particles with diameter of ca. 55 nm
- Infectivity can be neutralized by CD81-specific antibodies
- Infectivity can be neutralized by Ig from patients and anti-E2 Mab
- Cell culture-grown HCV is infectious \textit{in vivo} (chimp; chimeric mouse)
Virucidal efficacy of different alcohols against HCV

Quantitative suspension:

100 µl virus

900 µl disinfectant

mix, incubation, mix

titration infectivity (TCID_{50}/ml)
Effect of ethanol, 1-propanol and 2-propanol on HCV/BVDV

HCV

BVDV
HCV infectivity in comparison with HCV-RNA copy numbers

21°C
0, 7, 14, 28, 35 days

Titration infectivity (TCID$_{50}$/ml)
RNA isolation and quantification (qRT-PCR)

RNA copies (3x10$^7$)  RNA 0 days  RNA 21 days

Log$_{10}$ infectivity TCID$_{50}$/ml

Log$_{10}$ RNA copies/ml
HCV stability and inactivation in suspension

Ciesek et al. Journal of Infectious Diseases 2010
Steinmann et al. American Journal of Infection Control 2010
Steinmann et al. Antimicrobial Resistance and Infection Control 2013
Fig 1. Virucidal activity (reduction factors) of WHO formulations I (white columns) and II (black columns) against BVDV (A), HCV (B), poliovirus (C), adenovirus (D), and MNV (E) as a surrogate for human NoV following EN
Establishment of a HCV carrier assay

Dörrbecker et al. Journal of Infectious Diseases 2011
**Survival of dried HCV on inanimate surfaces**

![Graph showing survival of dried HCV on inanimate surfaces](image)

- **Log\(_{10}\) Infectivity TCID\(_{50}\)/ml**
- **Days**: 0, 1, 2, 3, 4, 5, 6, 7, 8
- **Conditions**: Control, 0.025, 0.25, 0.50, 0.025, 0.25, 0.50, 0.025, 0.25, 0.50%

- **Graph B**: Comparison between HCV-serum and HCV+serum
- **Graph C**: Cytotoxicity levels
- **Graph D**: Additional data on survival rates

The graph illustrates the survival rate of dried HCV on inanimate surfaces under various conditions, showing a decrease over time. The data points indicate that the infectivity TCID\(_{50}\)/ml decreases significantly over the 8-day period, with the control group showing a higher initial value compared to the treated groups.
Development of a drug transmission assay

In collaboration with NDRI, NY, USA Dr. Mateu-Gelabert, Prof. Hagan, Prof. Des Jarlais and Fixpunkt Hannover

[Graph showing Log_{10} RLU/well vs. temperature in °C]
Transmission of Hepatitis C virus among injecting drug users: viral stability and association with drug preparation equipment

![Graph showing viral stability over time](image)

- **Abcuvirine**: 500 µl
- **200 µl**: 200 µl
- **40 µl**: 40 µl
- **8 µl**: 8 µl
- **1.5 µl**: 1.5 µl

**Infectivity TCID₅₀/ml**

- **Container material**: virus spike
- **Recovery concentrated**: virus spike

![Images of drug preparation equipment](image)

**Dörrbecker et al. Journal of Infectious Diseases 2013**
Transmission of Hepatitis C virus among injecting drug users: viral stability and association with drug preparation equipment

Dörrbecker et al. Journal of Infectious Diseases 2013
HCV transmission by anaesthetica

Log\(_{10}\) infectivity ffu/ml

- control
- propofol

days

7  14  21  28  35

Gutelius et al. Gastroenterology 2010
Fischer et al. Clinical Infectious Diseases 2010
Steinmann et al. Clinical Infectious Diseases 2011
Behrendt et al. American Journal of Infection Control 2013
**Inactivation of HCV in blood products**

Methylene blue is a phenothiazine dye. Dyes of this class can enter the nucleic acid structure, and bind closely to the Guanosine residues of the DNA/RNA.

Following photoactivation in the region of 590 nm, the dye is able to chemically damage the genetic material, disrupting viral replication and infectivity.

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**Table: Reduction of HCV Infectivity**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\log_{10} \text{TCID}_{50} \pm \text{SD}$</th>
<th>$\log_{10}$ reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>spike bef. MB</td>
<td>$5.41 \pm 0.22$</td>
<td>0.12</td>
</tr>
<tr>
<td>0 J/cm$^2$ after MB</td>
<td>$5.29 \pm 0.16$</td>
<td>$\geq 2.26$</td>
</tr>
<tr>
<td>10 J/cm$^2$</td>
<td>$\leq 3.15$</td>
<td>$\geq 2.26$</td>
</tr>
<tr>
<td>20 J/cm$^2$</td>
<td>$\leq 3.15$</td>
<td>$\geq 2.26$</td>
</tr>
<tr>
<td>40 J/cm$^2$</td>
<td>$\leq 1.58$</td>
<td>$\geq 3.83$</td>
</tr>
<tr>
<td>120 J/cm$^2$</td>
<td>$\leq 1.58$</td>
<td>$\geq 3.83$</td>
</tr>
</tbody>
</table>

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**Table: Reduction of HCV Infectivity after Spiking**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\log_{10} \text{TCID}_{50} \pm \text{SD}$</th>
<th>$\log_{10}$ reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>after spiking</td>
<td>$5.61 \pm 0.54$</td>
<td></td>
</tr>
<tr>
<td>0.05 J/cm$^2$</td>
<td>$3.80 \pm 0.12$</td>
<td>$1.81$</td>
</tr>
<tr>
<td>0.1 J/cm$^2$</td>
<td>$2.81 \pm 0.12$</td>
<td>$2.80$</td>
</tr>
<tr>
<td>0.2 J/cm$^2$</td>
<td>$\leq 0.62$</td>
<td>$\geq 4.99$</td>
</tr>
</tbody>
</table>
Influence of tattoo ink on HCV infectiousness

Association of Tattooing and Hepatitis C Virus Infection: A Multicenter Case-Control Study

Kerrilynn Carney,1 Sameer Dhall,2 Ayse Aytaman,4,5 Craig T. Tenner,1,3 and Fritz Francois1,6

[Graphs showing reduction factors for suspension test and carrier test]
Thermo-stability of seven Hepatitis C virus genotypes in vitro and in vivo

Dörrbecker et al. Journal of Viral Hepatitis 2013
Inactivation of HCV and HIV by microwave?

Graphs showing the inactivation of HCV and HIV under different microwave powers and times. The graphs display the temperature in °C and the logarithm of the percentage of infection (relative to mock, non-treated) over time. The graphs include data from different strains: R2a - GT1a, R2a - GT2a, R2a - GT4a.
Inactivation of HCV and HIV by microwave?

Graph 1: Inactivation of HCV alone and in co-incubation with HIV at different microwave powers (90, 180, 360, 600, 800 Watt) over time (1, 2, 3 minutes).

Graph 2: Inactivation of HIV alone and in co-incubation with HCV at different microwave powers (90, 180, 360, 600, 800 Watt) over time (1, 2, 3 minutes).
Inactivation of HCV in human mother’s milk

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Conc. [mg/ml]</th>
<th>RF</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid</td>
<td>4.0</td>
<td>10</td>
<td>-0.17</td>
</tr>
<tr>
<td>Caproic acid</td>
<td>6.0</td>
<td>10</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>8.0</td>
<td>10</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Capric acid</td>
<td>10.0</td>
<td>5</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>12.0</td>
<td>5</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>14.0</td>
<td>20</td>
<td>0.96</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16.0</td>
<td>20</td>
<td>0.58</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18.0</td>
<td>20</td>
<td>1.08</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>16:1</td>
<td>2</td>
<td>≥ 2.46</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18:1</td>
<td>10</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>18:1</td>
<td>20</td>
<td>0.21</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2</td>
<td>5</td>
<td>3.46</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>18:3</td>
<td>5</td>
<td>≥ 3.71</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>20:4</td>
<td>1</td>
<td>≥ 3.71</td>
</tr>
</tbody>
</table>
Inactivation of HCV in human mother’s milk

Pfaender et al. Journal of Infectious Diseases 2013

Editorial Jhaveri: Protection against HCV and other enveloped viruses: “why breast is the best”
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