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High tolerance of hepatitis B virus to thermal disinfection

To the Editor:

Hepatitis B virus (HBV) is a severe global health burden, with approximately 2 billion infected individuals and more than 250 million carriers worldwide. Despite the availability of vaccines and therapeutics, chronic hepatitis B remains incurable. The virus is highly contagious and can circulate with 10^8 to 10^{10} infectious particles in a patient's blood. Because HBV has a very low minimal infectious dose of only 10 genomes, the transmission from only trace amounts of blood pose a serious risk for infection.[1] This risk highlights the importance of implementing guidelines for HBV prevention and management in infection control systems, such as thermal disinfection of medical devices. At present, the only relevant data available are from human studies conducted 50–70 years ago, which indicated that heating HBV specimens to 60 °C still resulted in productive infections in 50% of human volunteers.[2] Further, Krugman *et al.* inactivated 1:10 diluted HBV serum at 98 °C for 1 min and inoculated 29 mentally disabled children.[3] Their results initially provided no evidence of infection; however, in 1979, a more sensitive radioimmunoassay detected 3 sub-clinically infected individuals.[4] Later, Kobayashi *et al.* demonstrated inactivation of HBV 1:1,000 diluted in plasma at 98 °C for 2 min in chimpanzees.[5] Thus, cell culture models should be preferentially employed enabling accurate, rapid, and ethical determination of residual infectivity of HBV on a single-cell level for the definition of optimized thermal disinfection conditions. In the present study, we used a newly developed HBV cell culture system to thoroughly investigate the susceptibility of cell culture- and patient-derived HBV to thermal disinfection.[6]

Cell culture-derived HBV (HBVcc) particles were produced using stable HBV replicating HepAD38 cells. Patient-derived HBV characteristics are summarized in Table S1, all participants signed informed consent, and study procedures were approved under IRB No KC15TISI0951. To investigate the stability of HBVcc at different temperatures, HBV specimens stored at −80 °C were adjusted to 25 °C for 10 s before incubating at indicated temperatures ranging from 40° to 90 °C for 1, 2, 5, or 10 min in 0.2 ml thin-wall PCR tubes in a thermal cycler. After heat treatment, samples were immediately transferred to ice before inoculation of HepG2-NTCPsec+ cells. For autoclaving, samples were heated to 121 °C for 20 min. Detection of single HBV-infected cells was achieved by immunofluorescence analysis (IFA) of inoculated HepG2-NTCPsec+ cells (see also supplementary materials and methods).

With increasing temperatures, infectivity of HBVcc declined but was still detectable when exposed to 80 °C for 10 min and to 90 °C for 5 min (Fig. 1A-B). The Z-value (thermal death time), defined as the number of degrees the temperature has to be increased to achieve a 10-fold decrease in decimal reduction time (D-value), was calculated as 10.87 (Fig. 1B). Incubation of HBVcc at 98 °C for 1 or 2 min revealed detectable infection, while complete abrogation of infectivity was achieved only at longer incubation times or after autoclaving the virus samples (Fig. 1C, upper panel, and 1D, right). Next, the inactivation profiles of 3 different clinical HBV isolates were investigated. We used 2 HBV patient isolates with high viral genome loads and one with a lower viral genome load (Table S1). For patient 1-derived HBV, infection events were detected after 1 min of incubation, however, were negative at 2, 5, and 10 min at 98 °C incubation times (Fig. 1C, lower panel, and 1D, right). For the other 2 HBV patient-derived isolates (patient 2 and 3) infected cells could be detected even after incubation at 98 °C for 1 and 2 min (Fig. 1E-H), which is in line with the cell culture-derived HBV particles. Finally, we followed the propagation of patient 2 and 3 HBV isolates, incubated at 98 °C (0, 1, 2, 5, and 10 min), during long-term infection experiments between 2 to 5 weeks post-inoculation. For patient 2, the virus propagated by 2–3.2-fold under untreated conditions as well as after incubation for 1 and 2 min at 98 °C (Fig. 1E-F). Comparable results were observed for patient 3, however, HBV propagation rate was decreased to 1.7–2-fold, which is likely due to the 500-fold lower viral load in the serum of this patient (Fig. 1G-H). Virus growth with unchanged and typical slow kinetics could be observed for the samples treated for 1 and 2 min at 98 °C (Fig. 1E-H), demonstrating that high temperature-treated HBV particles had the ability to spread in tissue culture. Important to note, no virus infection was detectable with virus samples treated at 98 °C for ≥5 min at 2 or 5 weeks of propagation.

Thermal disinfection strategies play an important role in infection control, especially for cleaning and decontamination of reusable medical equipment. Per current guideline (EN ISO 15883), high temperatures are recommended for processing medical devices by thermal disinfection to inactivate stable pathogens like HBV.[7] Our investigation of HBV's susceptibility to heat demonstrated an even higher level of tolerance. Therefore, we propose that established disinfection guidelines should be followed very strictly and in order to completely inactivate HBV, these data suggest that virus inactivation should be at a temperature of 98 °C or higher for a minimum of 5 min.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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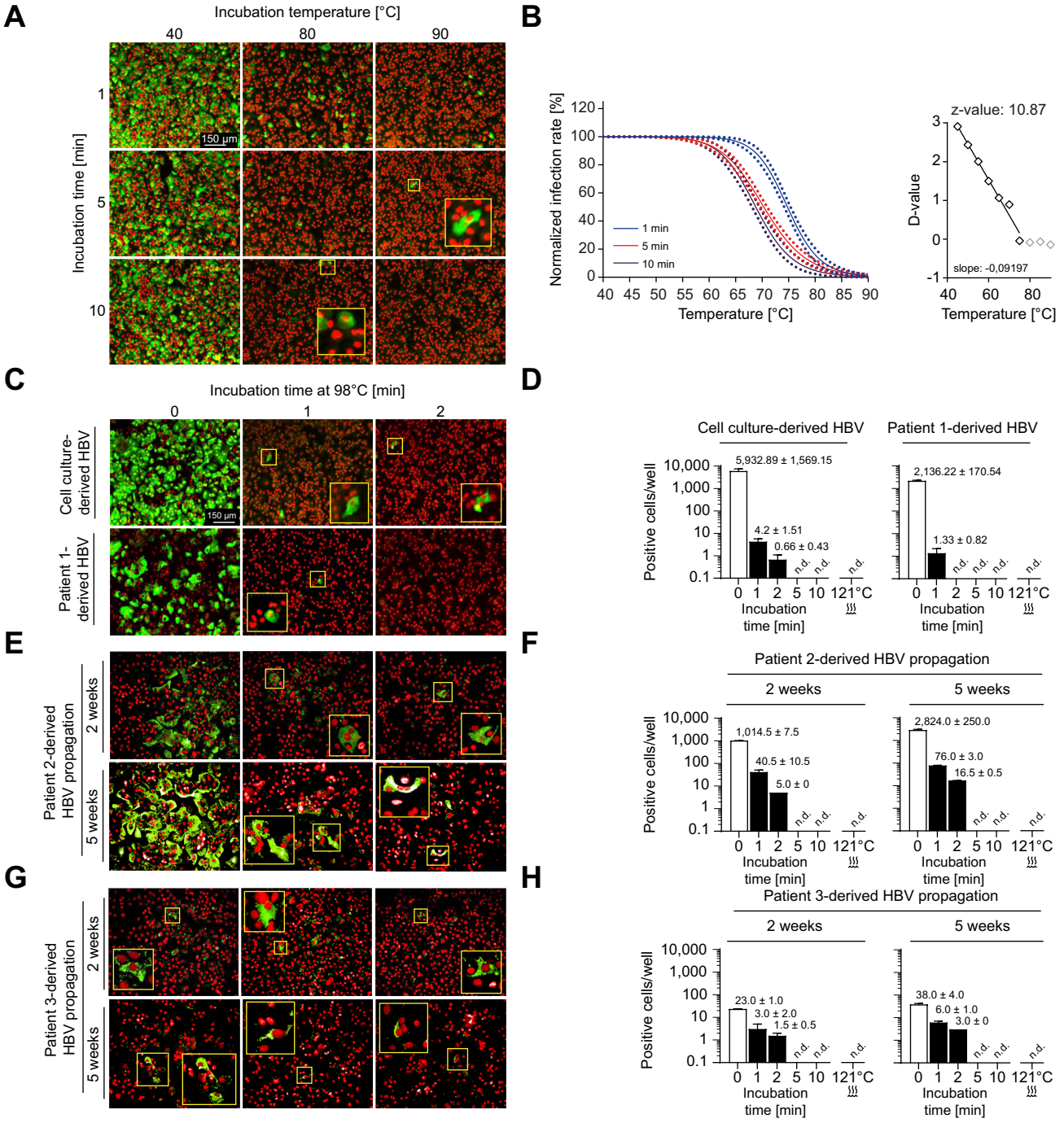

Fig. 1. Heat treatment of cell culture and patient-derived HBV. (A) Cell culture-derived HBV particles were incubated for 1, 5, and 10 min at indicated temperatures, followed by inoculation of HepG2-NTCPsec+ cells. At 1 week post-inoculation, HBV infection was determined by HBcAg-specific immunofluorescence analysis (IFA). (B) Temperature-response curves of HBV infection rates were normalized to 1 min at 40 °C. Normalized numbers of infected cells after 1 min (blue line), 5 min (red line), or 10 min (purple line) at respective temperatures (left panel, n = 3) are shown. The thermal death time (Z-value) was calculated as the slope of linear regression of the decimal reduction time (D-value) at respective temperatures. (C) Cell culture and patient 1-derived HBV particles were incubated at 98 °C for 0, 1, 2, 5, or 10 min, followed by inoculation of HepG2-NTCPsec+ cells and HBV infectivity measurement via IFA. (D) Average absolute numbers of HBV-positive cells per entire well of a 384-well plate at indicated 98 °C incubation times or after autoclaving at 121 °C. Values shown are HBV-infected cell numbers ± standard deviation (n = 3). (E) High titers of patient 2-derived HBV particles were incubated at 98 °C for 0, 1, 2, 5, or 10 min, followed by inoculation of HepG2-NTCPsec+ cells and propagation of HBV progeny over 2 or 5 weeks before infectivity measurement via IFA. (F) Average absolute numbers of HBV-positive cells per well of a 384-well plate at indicated 98 °C incubation times at 2 and 5 weeks of virus propagation. Values shown are HBV-infected cell numbers ± standard deviation per entire well of a 384-well plate (n = 2). (G) Low titers of patient 3-derived HBV particles were incubated at 98 °C for 0, 1, 2, 5, or 10 min, followed by inoculation of HepG2-NTCPsec+ cells and propagation of HBV progeny over 2 or 5 weeks before determination of infection via IFA. (H) Average absolute numbers of HBV-positive cells per entire well of a 384-well plate at indicated heat incubation times at 2 and 5 weeks of virus propagation. Values shown are HBV-infected cell numbers ± standard deviation per entire well of a 384-well plate (n = 2).

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.08.022.

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Author names in bold designate shared co-first authorship

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Alexander König[1,#]
Thoa Thi Than[1,#]
Daniel Todt[2]
Seung Kew Yoon[3]
Jochen Steinmann[4]
Eike Steinmann[2,*]
Marc P. Windisch[1,*]

[1]*Applied Molecular Virology Laboratory, Unmet Medical Needs Division, Institut Pasteur Korea, 696 Sampyung-dong, Bundang-gu, Seongnam-si 13488, Gyeonggi-do, South Korea*
[2]*Ruhr University Bochum, Faculty of Medicine, Department for Molecular and Medical Virology, 44801 Bochum, Germany*
[3]*Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 06591 Seoul, South Korea*
[4]*Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, 28259 Bremen, Germany*

*Corresponding authors. Address: Applied Molecular Virology Laboratory, Discovery Biology Department, Institut Pasteur Korea, Gyeonggi-do 463-400, South Korea. Tel.: +82 31 8018 8181; fax: +82 31 8018 8014 (M. Windisch). Department of Molecular and Medical Virology, Ruhr-University Bochum, Universitätsstr. 150, 44801 Bochum, Germany. Tel.: +49 234 32 23189; fax: +49 234 32 14352 (E. Steinmann).
E-mail addresses: steinmann.eike@ruhr-uni-bochum.de, marc.windisch@ip-korea.org

[#] Alexander König and Thoa Thi Than contributed equally to this work.

Pitfalls in measuring temporal trends for late diagnosis of viral hepatitis

To the Editor:

To monitor the prevention and treatment of chronic diseases, the World Health Organization and others report age-standardized death rates, years of life lost, and disability-adjusted life years.[1] However, these measures do not capture information on how late in their disease course patients first present for care. This information can be useful to gauge the adequacy of disease screening efforts. Clinical stage at presentation, as used in oncology research, can be used as a measure of late diagnosis,[2,3] but the detailed clinical information used to stage liver disease is lacking from health administrative data sources, which are increasingly used to study the real-world outcomes of patients with liver disease.[4,5]

Comparing the time of diagnosis to the time of development of a complication may be an indirect way to determine the proportion of individuals who are inadequately served by current disease screening efforts. When diagnosis and complications occur very close together in time, inferences can be made about the clinical stage at diagnosis. Two previous studies have reported on late diagnosis of hepatitis B and C infection in Canada and Australia.[6,7] Both studies included plots of the proportion of patients with late diagnosis over time, and reported that late diagnosis was becoming less common. Because of the potential policy importance of such findings, we attempt here to illustrate how the choice of methods used to identify late diagnosis can determine the direction of observed trends. It is essential to consider possible sources of bias in this measurement, to avoid making erroneous inferences.

Methods and results

We undertook a cohort study of patients diagnosed with hepatitis C virus (HCV) infection based on laboratory tests in Ontario between 2000 and 2014. Using data from Public Health Ontario, we identified all patients with positive anti-HCV antibody or HCV RNA. We excluded patients who had a positive antibody but negative HCV RNA, signalling a previous (now cleared) infection. We also excluded those with an HCV diagnosis before January 1st 2000 or within 1 year of their earliest health insurance eligibility. Because we relied on health administrative data to detect outcomes, we excluded those whose HCV testing data could not be linked to health administrative data holdings. Ontario health administrative data were accessed in accordance

Keywords: Viral hepatitis; Hepatitis B virus; Hepatitis C virus; Bias; Temporal trends; Late diagnosis.