

Correspondence

Efficiency of Hepatitis C Virus Infection *in Vitro*

To the Editor-in-Chief:

In the scientific article from Lázaro et al¹ entitled *Hepatitis C Virus Replication in Transfected and Serum-Infected Cultured Human Fetal Hepatocytes*, the authors provide convincing evidence for the successful infection of human fetal hepatocytes with hepatitis C virus (HCV) *in vitro*. However, the infection occurred after the overnight incubation of the cells with HCV-infected patient sera.

This infection time could be dramatically reduced to 1 hour by following an infection procedure consisting of the removal of the cell-bound lipoproteins before the addition of the viral inoculum onto the cells.^{2,3} In this infection procedure, the removal of cell-bound lipoproteins from the low-density lipoprotein (LDL) receptor might be the crucial step for efficient hepatitis C virus infection *in vitro* (Figure 1).

The conventional "state-of-the-art" model for the replication of HCV in tissue culture is based on the use of established hepatocyte cell lines, such as the HepG2 and the Huh-7 cell lines. This method uses the transfection

of *in vitro*-transcribed hepatitis C virus RNA replicons in the hepatoma cell line Huh-7. However, this transfection procedure does not represent by any means an infection model. Although replicon-based assays do provide an *in vitro* subgenomic replication system, which is precious for the screening of antiviral molecules, it is not relevant for the study of the early steps of infection and the evaluation of adsorption and internalization for the sake of receptor evaluation or for neutralization studies and prophylaxis. Overnight incubation of cells with human sera is also not a reproductive procedure of infection with HCV.

Therefore, it is very surprising that the dextran sulfate infection procedure has not been chosen as a method of choice, yet, and that it is not more referenced in scientific articles. This very convenient method is based on the evident scientific literature cited in the references of the two above-mentioned articles. To reveal the newly synthesized HCV RNAs by reverse transcription-polymerase chain reaction, it is obvious that care has to be taken to completely remove dextran sulfate, since the latter compound might inhibit the further polymerase chain reaction.

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References

1. Lázaro CA, Chang M, Tang W, Campbell J, Sullivan DG, Gretch DR, Corey L, Coombs RW, Fausto N: Hepatitis C virus replication in transfected and serum-infected cultured human fetal hepatocytes. *Am J Pathol* 2007, 170:478–489
2. Favre D, Berthillon P, Trepo C: Removal of cell-bound lipoproteins: a crucial step for the efficient infection of liver cells with hepatitis C virus *in vitro*. *C R Acad Sci III* 2001, 324:1141–1148
3. Favre D, Muellhaupt B: Potential cellular receptors involved in hepatitis C virus entry into cells. *Lipids Health Dis* 2005, 4:9

Author's reply:

We thank Dr. Favre for calling our attention to the dextran sulfate infection procedure that they have described. We were not aware of this technique but realize that it would be a significant technical advance if HCV infection can be achieved after only 1 hour of exposure to HCV-infected serum.

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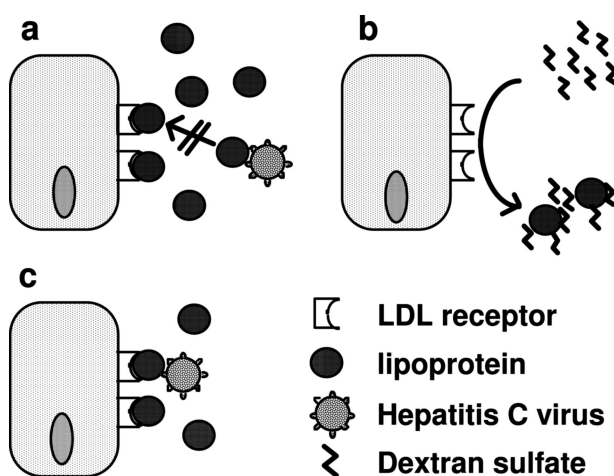


Figure 1. **a:** The binding of the HCV-lipoprotein complex to the LDL receptor is hampered *in vitro* by the cell-bound lipoproteins and by the vast excess of free lipoproteins present in the human blood. **b:** Before HCV infection, the cell-bound lipoproteins are removed from the LDL receptor by using dextran sulfate, thus generating free LDL receptors. **c:** Lipoprotein-free LDL receptors can bind the HCV-lipoprotein complex, thus allowing adsorption and penetration of HCV into target cells. Reprinted from *C R Acad Sci III*, 324, Favre D, Berthillon P, Trepo C, Removal of cell-bound lipoproteins: a crucial step for the efficient infection of liver cells with hepatitis C virus *in vitro*, 1141–1148, Copyright (2001)² with permission from Elsevier.