



Viral RNA extraction from Infected Tissues at Leuven University

CONTEXT

In the context of one of the laboratories at the University of Leuven (Belgium), antiviral chemotherapy is studied and focused on.

To evaluate the *in vivo* activity of the newly discovered antiviral compounds, we treated virus-infected mice with the antiviral compound, and extract the organs in which the virus replicates.

MATERIAL

- Precellys®24
- Precellys® kit CK28 (big ceramic beads)
- Sample : 30-75mg of murine pancreas
- Buffer : volume is adjusted in order to get a 5% w/v homogenate

PROTOCOL

- Precellys®24 parameters:
6500 rpm, 3x5 sec.



CONCLUSION

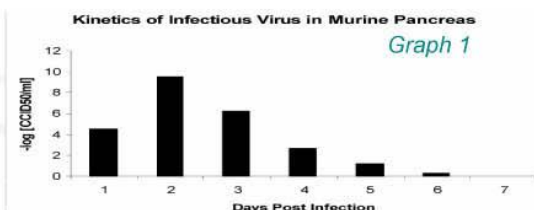
The Precellys®24 helped us to evaluate the *in vivo* activity of the newly discovered antiviral compounds. In this study, the difference between (1) and (2) is that some viruses keep their genome in a host cell without actively replicating (i.e. they stay in their "latent" condition).

RESULTS

After homogenizing the murine pancreas, Leuven University detects :

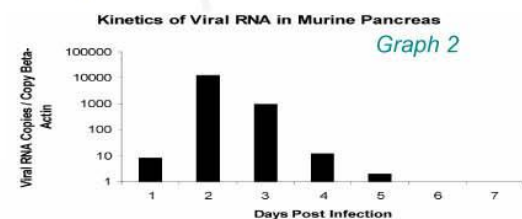
- Firstly the viral RNA (1)

The homogenization in lysis buffer RLT is performed, and worked further with the supernatant to extract the viral RNA. Graph (1) presents the evolution of viral RNA in the pancreas of viral-induced mice. It is expressed as "copies viral RNA per copy beta actin" (RT-QPCR after total RNA extraction).



- Secondly replicating the infectious virus(2)

In the second try (2) the homogenization in growth medium, (MEM, Gibco), is performed. Using lysis buffer in this try would also lyse the viral particles so they would lose their infectivity. The supernatant is then used to titrate on a cell culture (96-well plates, and the titer of the virus is determined (expressed as CCID50).



Graph(2) presents the evolution of infectious virus: the titer of infectious virus is expressed as "log CCID50/ml" where CCID50 stands for "Cell Culture Infective Dose 50%", which is a measure to express the titer of a virus stock.

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