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Inactivation of HIV-1 using Pressure Cycling Technology (PCT)

Janet L. Lathey*, Bita Nakhai, Pinar Tuzmen, Feng Tao, Richard T. Schumacher, Mark M. Manak*, and Nathan P. Lawrence

PCT Group and *Biotech Research Labs, Boston Biomedica Inc., 217 Perry Parkway, Gaithersburg, MD

Contact Information:

Janet L. Lathey, Ph.D.
Director of Virology/Immunology
BBI Biotech Research Laboratories
217 Perry Parkway
Gaithersburg, MD 20877

Phone 301-208-8100 ext. 191
Fax 301-208-8829
jlathey@bbii.com

Abstract

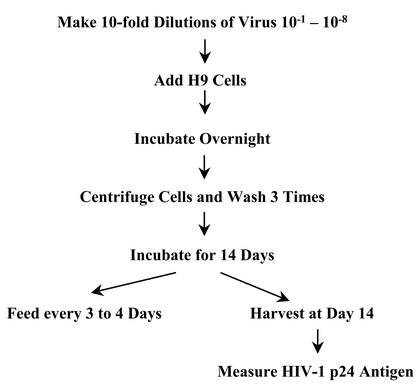
Background: Viral inactivation is critical in the preparation of biological products, thus new and better methods for inactivation are always in demand. To this end, a new procedure called Pressure Cycling Technology (PCT) was used to inactivate HIV-1 and compared to conventional laboratory procedures.

Methods: For this study, a pressure cycling instrument (Barocycler™) developed by Boston Biomedica, Inc. (BBI), designed to cycle pressure between ambient and 80 kpsi, was used to process samples. Treatment of HIV-1 was performed in 2 matrices, Basematrix (defibrinated plasma) and tissue culture medium (RPMI). Inactivation was scored as reduction in viral titer by infectivity assays measuring $TCID_{50}$ on susceptible cells. Viral inactivations by multiple freeze-thaws or treatment with 0.1% BPL were used as controls. HIV-1 p24 antigenicity was also measured by ELISA.

Results: In one series of experiments using RPMI, the effect of the number of pressure cycles applied between ambient and 80 kpsi resulted in an inactivation dose response directly related to an increase in the number of cycles. Log_{10} reduction was 3.8, 4.6, 5.3, and 6.0 for 8, 20, 35, and 50 cycles, respectively. In other experiments, the number of cycles was held constant at 8, while the pressure was varied from ambient to points between 30 to 80 kpsi. Under these conditions, there was little change in viral inactivation (2.2-2.7 Log_{10}). An inactivation dose response curve was also observed with increasing the number of cycles with HIV-1 in Basematrix; however, the inactivation was less than that observed with HIV-1 in RPMI. Log_{10} reduction was 1.3, 1.5, and 2.3 for 4, 8, and 20 cycles, respectively. Controls were also evaluated, and in comparison, 8 freeze thaws inactivated HIV-1 only 1 Log_{10} and 0.1% BPL at least 3 Log_{10}. At conditions that inactivated the greatest amount of virus, there was no significant reduction in p24 antigen.

Conclusion: PCT is an effective method for HIV-1 inactivation. By increasing the number of pressure cycles to 50, HIV-1 infectivity was reduced by 6 Log_{10}. In addition, even at the highest number of cycles evaluated (50); the inactivated virus retained its HIV-1 p24 antigenicity.

HIV Infectivity Assay



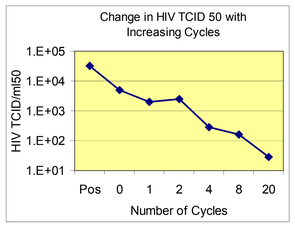
Novel Method in Infectious Agent Inactivation using PCT

Experimental Method:

- Test samples were packaged in EVA plastic bags and thermally sealed
- Pressure treatments were conducted with the Barocycler V2.5a shown below (BBI Source, Garden Grove, CA). Temperature of the reaction chamber (blue cylinder in the picture) was controlled with a circulating chiller.
- After the pressure treatment, titer of the HIV virus was determined and compared with controls, i.e. samples that were processed in parallel, but without cyclic pressure treatment.

Fig. 1. Barocycler V.2.5a
Pressure: ambient to 80 kpsi or 537 MPa
Pressure rise time: < 10 sec.
Pressure release time: < 3 sec.
Temperature: between - 40 and 70 °C
Sample volume: up to 5 mL

Results



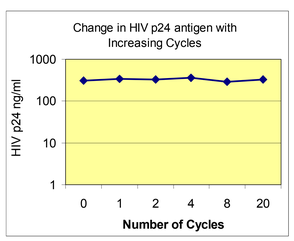
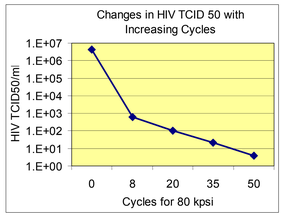


Figure 2. Inactivation of HIV-1 under Conditions of Constant Pressure and Increasing Number of Cycles. HIV stock virus was diluted 1:5 in Basematrix (difibrinated plasma) and subjected to 0, 1, 2, 4, 8, or 20 rounds of pressure cycling at 80 kpsi or 537 MPa. Level of HIV-1 inactivation was evaluated by infectivity assay determining $TCID_{50}$ (Tissue Culture Infectious Dose that infects 50% of cultures). Conservation of antigenic structure was determined by HIV-1 p24 antigen ELISA with a read out in ng/ml. Infectivity decreased with increasing number of cycles with a reduction of 2.3 log_{10} with 20 pressure cycles. The HIV-1 p24 concentration remained constant throughout the 20 cycles.



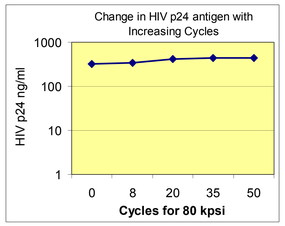
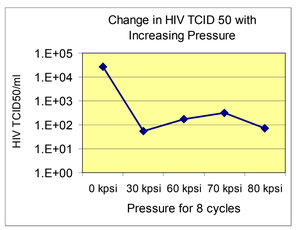


Figure 3. Inactivation of HIV under Conditions of Constant Pressure and Increasing Number of Cycles. HIV-1 stock virus was subjected to 0, 8, 20, 35, or 50 rounds of pressure cycling at 80 kpsi. Level of HIV-1 inactivation was evaluated by infectivity assay determining $TCID_{50}$. Conservation of antigenic structure was determined by HIV-1 p24 antigen ELISA. Infectivity decreased with increasing number of cycles with a reduction of 5.3 log_{10} with 50 pressure cycles. The HIV-1 p24 concentration remained constant throughout the 50 cycles.



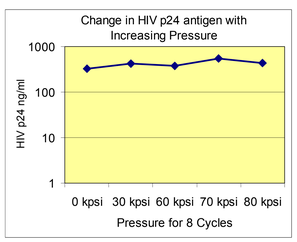
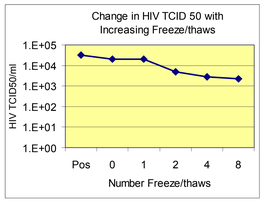


Figure 4. Inactivation of HIV-1 under Conditions of Increasing Pressure and Constant Number of Cycles. HIV-1 stock virus was subjected to 0, 30, 60, 70, or 80 kpsi of pressure for 8 cycles. Level of HIV inactivation was evaluated by infectivity assay determining $TCID_{50}$. Conservation of antigenic structure was determined by HIV-1 p24 antigen ELISA. Infectivity decreased at 30 kpsi, then remained constant with increasing pressure with a reduction of 2.7 log_{10} at 30 kpsi. The HIV-1 p24 concentration remained constant with increasing pressure.



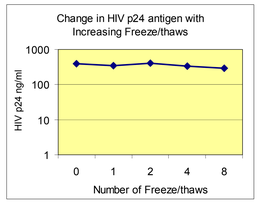


Figure 5. Inactivation of HIV-1 with Multiple Freeze/thaws. HIV stock virus was diluted 1:5 in Basematrix and subjected to 0, 1, 2, 4, or 8 rounds of freezing and thawing at -20ºC. Level of HIV inactivation was evaluated by infectivity assay determining $TCID_{50}$. Conservation of antigenic structure was determined by HIV-1 p24 antigen ELISA. Infectivity decreased very little with increasing freeze/thaw with a maximum reduction of 1 log_{10}. The HIV-1 p24 concentration remained constant throughout the 8 freeze/thaws.

β-Propiolactone (BPL) Treated HIV-1. HIV-1 stock virus was diluted in Basematrix and treated with 0.1% BPL. The level of inactivation was then evaluated by infectivity assay determining $TCID_{50}$ of BPL-treated and untreated stocks. Untreated HIV-1 stock had a $TCID_{50}$ of 3.87 log_{10} compared to a $TCID_{50}$ of < 0.75 log_{10} when treated with 0.1% BPL, >3 log_{10} reduction.

Table 1. Hypothetical mechanisms of action in PCT inactivation.

	CAUSE	HYPOTHETICAL EFFECT
I	Solution under subzero temperature, cyclic pressure conditions	Rapid freeze-thaw; formation of ice crystals and cause cell damages, e.g. in virus
II	Thermodynamic effects of low temperature, and high pressure	Protein denaturation and disruption of biomolecular interactions, e.g. disassociation of multimeric proteins and viral particles
III	Rapid pressure change	Microbubble collapse to cause localized cavitation and viral damages
IV	Changes in permeability of cell membrane or viral particle	Transient or permanent exposure of proteins or nucleic acids to extracellular enzymes or inhibitors
V	Pressure cycling and dynamic enhancement	Pressure cycling has beneficial effects on both the rate of inactivation and recovery of protein activity

Summary

Pressure Cycling Technology process with a Barocycler™ is an effective method for inactivating HIV-1 that does not require the addition of toxic chemicals.

- A 6 log_{10} reduction in HIV-1 infectivity can be achieved without changing the antigenicity of p24 antigen.
- Increasing the pressure cycles to which HIV-1 is exposed increases the inactivation. Most of the inactivation occurs within the first 20 cycles.
- Increasing the level of pressure greater than 30 kpsi had little effect on inactivation.
- Inactivation appeared to be more effective in tissue culture medium than Basematrix; 4.6 log_{10} compared to 2.3 log_{10} reduction, respectively.
- Inactivation with PCT was more effective than multiple freeze/thaws and as good or better than BPL.

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