Isolate and concentrate viruses for improving virus culture

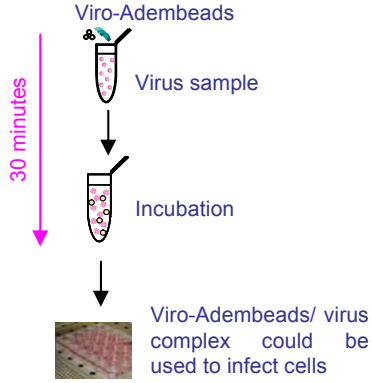
Virus culture remains the only way to detect infectious virus, to analyze clinically relevant viral phenotypes or to isolate unknown pathogens. Although viral isolation and detection by *in vitro* culture have been improved by centrifugation techniques, these methods remain time consuming and labor intensive, and some pathogens are difficult to be grown. Ademtech has developed an innovative tool to speed and facilitate virus culture, and increase virus infection efficiency.

By Dr Elise Flavigny
ADEMTECH SA

Introduction

Viro-Adembeads are specifically developed magnetic particles to capture viruses from biological samples. Following incubation in a virus containing sample, Viro-Adembeads associated to viruses are recovered and can be directly used to infect target cells. Compared to conventional infection protocols, Viro-Adembeads drastically increase infection efficiency ; this phenomenon is thought to result both from the concentration effect mediated by virus capture on Viro-Adembeads, and from favouring cell-virus contact by beads sedimentation that increases virus concentration at the very vicinity of target cell.

Figure 1 : Virus capture procedure



Material and Methods

Infection, culture and titration (table 1) :

HIV-1 was captured with Viro-Adembeads as described in the product protocol (Figure 1). Immediately after capture, Virus/beads complex was incubated with PHA-stimulated PBMC. The virus infection was monitored in comparison with two “controls” : the initial virus solution and centrifugation conventional techniques. Indeed, HIV-1, was prepared by centrifugation at 17 000g for 1h at 4°C. The inoculum and cells were pre-incubated for 1h30 at 37°C.

CMV and Influenza A were captured with Viro-Adembeads as described in the product protocol . For CMV and Influenza A, inoculum (Viro-Adembeads or initial solutions) was directly added to confluent monolayer of MRC-5 and MDCK cells respectively. Titration of Influenza A was performed by hemagglutination (HA). HA-titer is the highest dilution of supernatant that resulted in agglutination.

Quantitative RT-PCR and PCR analysis:
HIV-1 quantitation was performed by quantitative Real Time RT-PCR from RNA extracts on the ABI-PRISM 7000 Sequence Detection System using the TaqMan reaction system. CMV was detected and quantified by Real Time PCR performed from DNA extracts on the Light Cycler instrument using TaqMan reaction system. External standard curves were generated from 6 log10 HIV-1 RNA or CMV plasmid standard reference serial dilutions.

Virus	Cell type	Source
HIV-1	PBMC	Human
CMV	MRC-5 Lung fibroblasts	Human
Influenza A	MDCK Kidney epithelial cells	Canine

Table 1 : Capture and Culture of viruses

Viro-Adembeads capture and concentrate virus

Capture virus from biological samples

Viro-Adembeads capture independently of specific viral proteins or epitope. Virus capture is based on direct electrosteric interactions with Viro-Adembeads via. Following incubation in various virus-containing medium, Viro-Adembeads can efficiently capture various viruses (Table 2). For HIV-1, higher capture was observed in culture supernatant versus plasma sample suggesting some interferences with plasmatic proteins.

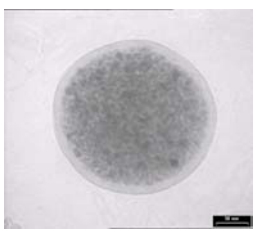
Virus, culture medium	Titer*	Capture efficiency
HIV-1, culture supernatant	10^5	99 %
HIV-1, human plasma	10^5	70 %
CMV, culture supernanant	10^5	86 %
CMV, culture supernatanrt	10^4	**99 %**
CMV, culture supernatant	10^3	86 %
Influenza A (H1N1) , culture supernatant	ND**	Yes (ND)
Rotavirus, feces	ND	Yes (ND)

Table 2 : Capture efficiency was determined by quantitation of viral amount in supernatant following capture by Viro-Adembeads compared to viral amount in starting sample.* initial virus was quantitated by RT-PCR for HIV, PCR for CMV, Hemaglutination for Influenza A.** Positive samples used were identified by qualitative Elisa assay.

Concentrate alive and infectious virus

The infectivity of captured virus was determined by using 3 different models. Viro-Adembeads capture was performed from medium inoculated with Influenza A (H1N1), CMV or HIV-1. An increase of the viral concentration was observed compared to initial virus solution (Table 3, Figure 4).

Figure 2 : Viro-Adembeads



CMV	PFU
Initial virus solution	200
Capture with Viro-Adembeads	>500

INFLUENZA A	HA titer		
Initial virus solution	1/16	<1/4	<1/4
Capture with Viro-Adembeads	1/32	1/16	1/4-1/8

Table 3 : CMV titration by MRC-5 infection and H1N1 titration by HA following infection of MDCK Cells with 3 serial dilution of virus solution stock.

Viro-Adembeads increase and accelerate HIV-1 production

HIV-1 spiked into culture medium or plasma were captured with Viro-Adembeads prior to PBMC infection. HIV-1 replication was followed over 11 days. Viro-Adembeads increase and accelerate HIV-1 production in comparison with the infection performed with the initial virus solution (Figure 3). Finally, Viro-Adembeads provide gentle method that preserve virus infectivity.

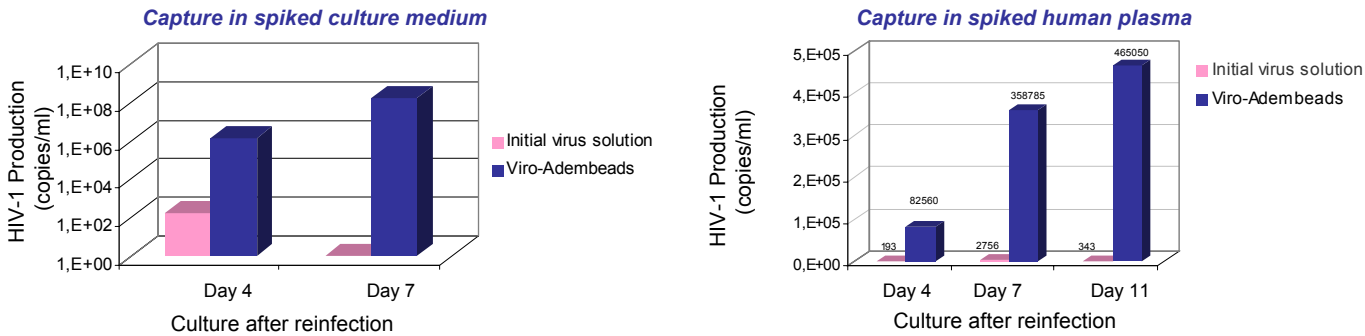


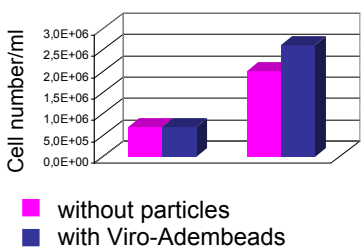
Figure 3: HIV-1 production assessed by quantitative RT-PCR from culture supernatant following infection with control initial virus solution and with Viro-Adembeads.

Evaluation of Viro-Adembeads vs conventional methods

Viro-Adembeads facilate and improve viral culture

HIV-1 concentration from plasma of infected patients is usually carried out by centrifugation or ultracentrifugation. However these methods are laborious and time consuming (2-3 hours) and may damage the viruses. HIV-1 spiked into plasma (10^5 copies/ml) were incubated with PBMC cultures. HIV-1 was concentrated with Viro-Adembeads or with centrifugation method prior to cell infection. Infection efficiency was evaluated by quantification of viral production in culture supernatant. As shown in Figure 4, Viro-Adembeads are more efficient than centrifugation (10 fold higher). Same results were obtained using ultracentrifugation technique for virus concentration. The lower culture efficiency after processing by centrifugation, probably reflects gel formation or plasma clotting. The presence of residual antiviral factors (such as neutralizing antibodies, complement, cytokines, drugs) may inhibite virus infection. Besides, virus aggregation may damage the virus.
HIV plasma culture is facilitated by the use of Viro-Adembeads. We have also observed that the complex virus/magnetic particles increases the sedimentation rate of the viral particles and therefore aid the virus attachment and subsequent infection process.

Figure 5 : PBMC culture in the presence of Viro-Adembeads.



No toxicity was observed. Viro-Adembeads are fully compatible with cell culture.

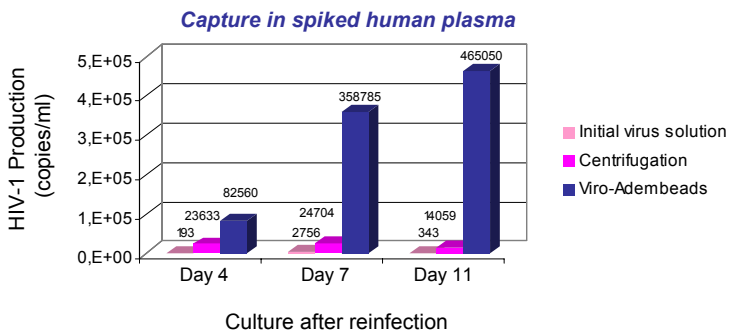
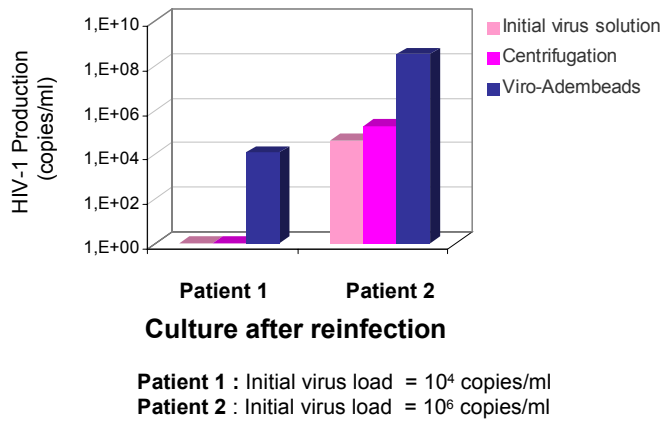


Figure 4: HIV-1 spiked plasma culture: HIV-1 production assessed by quantitative RT-PCR from culture supernatant following infection with control initial sample and with Viro-Adembeads vs centrifugation control.

Evaluation of Viro-Adembeads vs conventional methods

Higher Sensitivity

The capture efficiency directly from HIV-1 infected plasma patients was determined (Figure 6). These results showed that the use of Viro-Adembeads allow to detect HIV in plasma that could not be detected by conventional centrifugation techniques. Thanks to biomagnetic processing, virus particles are bound to magnetic particles that allow quick and reliable separation of virus from plasma. Thereby, the infection process occurs in the absence of patient plasma and potentially inhibitors.



Patient 1 : Initial virus load = 10^4 copies/ml
Patient 2 : Initial virus load = 10^6 copies/ml

Figure 6: HIV infected patient plasma culture: HIV-1 production assessed by quantitative RT-PCR from culture supernatant following infection with initial virus isolation, Viro- with Adembeads vs centrifugation control.

Higher Reproducibility

HIV-1 culture using Viro-Adembeads is more reproducible than conventional centrifugation techniques (Figure 7). As expected, the capture efficiency after centrifugation varied among the 5 samples tested. The variation among the 15 samples using Viro-Adembeads is less important.

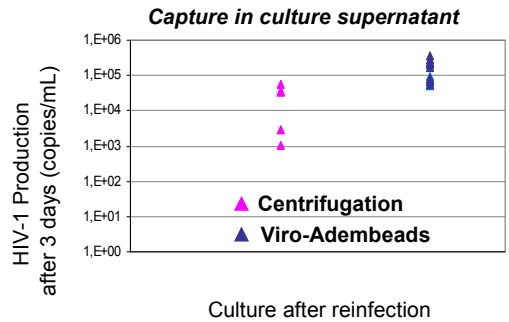


Figure 7: HIV-1 spiked culture medium: HIV-1 production assessed by quantitative RT-PCR from culture supernatant following infection with Viro- Adembeads vs centrifugation control.

Viro-Adembeads for capture and virus culture benefits

- **Capture and concentrate virus**
- **Simple and fast: 30 minutes**
- **Gentle virus separation method: isolates alive and infectious virus**
- **Viro-Adembeads increase and accelerate virus production**
- **Higher sensitivity**
- **Higher reproducibility**

Product	Size	Code number
Viro-Adembeads For virus capture and culture	25 prep	07010