

Viro-Adembeads Efficiency capture Flu viruses.

In recent publications, Dr Akikazu Sakudo and colleague of the Research Institute for Microbial Disease of Osaka University developed a new method using magnetic particle to facilitating the isolation of infectious viruses. The possible emergence of pandemic influenza virus has become a serious threat. The monitoring of infectious virus will be crucial for further virological analysis and the development of vaccines. However, conventional methods are relatively time-consuming, reduce infectivity and are incompatible with viral detection methods.

These studies highlight the use of Viro-Adembeads for the capture of Human and Avian Influenza viruses.

Influenza infection

Influenza Virus are a common seasonal disease that occur mainly in winter, and affect respiratory tract. Human influenza viruses causes also severe upper respiratory tract infections in infants, young children and elderly with an estimated to five millions cases of severe illness and 250,000-500,000 deaths per year worldwide.

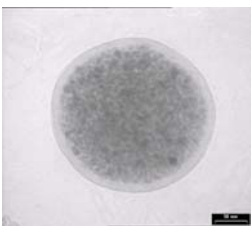


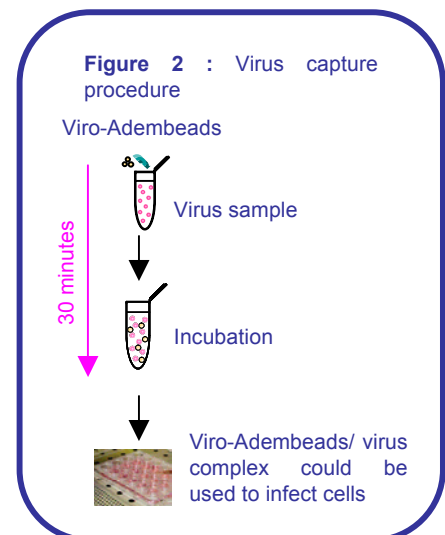
Figure 1 : Viro-Adembeads

Experimental Approach

Traditional method, polyethyleneglycol (PEG) precipitation and ultracentrifugation, are time-consuming, reduce infectivity and incompatible with conventional viral detection methods. Ultracentrifugation reduces infectivity and increases false positive rate combined with PCR. PEG precipitation is simple but partially inactivates infectious viruses. Another different approach is to use magnetic beads to simplify the process.

Viro-Adembeads are uniform and monosize magnetic nanoparticles with a large and well defined area that ensure optimal reproducibility, capacity and performance. Viro-Adembeads capture virus via a proprietary anionic and bioadhesive polymer (Fig.1).

Viral capture was performed as described in Fig.2. Following incubation in a virus containing sample, Viro-Adembeads associated to viruses are recovered and can be directly used to infect target cells.



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

Sucessful recovery of infectious Human and Avian Influenza viruses

Viro-Adembeads capture Human Influenza Viruses

There are three types of seasonal influenza – A, B and C based on the antigenicity of nucleoprotein and matrix protein. Type A influenza viruses are further typed into subtypes according to different kinds and combinations of virus surface proteins: hemagglutinin and neuraminidase. Among many subtypes of influenza A viruses, Influenza A/H1N1 and A/H3N2 subtypes are currently circulating among humans. Influenza viruses circulate in every part of the world. Sixteen HA subtypes (H1 to H16) and nine NA subtypes (N1 to N9) have been identified.

Dr Sakudo and colleague showed by immunochromatography and hemagglutination assay that influenza A virus (H1N1) was captured by the Viro-Adembeads from cell culture medium of MDCK cells infected with influenza A virus, allantoic fluid and nasal aspirates. This method is applicable for a large range of strains (A/H3N2, B/Yamagata-like, B/Victoria-like). Successful captures have been demonstrated using 5 H1N1 influenza A viruses, 10 H3N2 influenza viruses and 6 influenza B viruses. Hemagglutination assay showed that Viro-Adembeads efficiently concentrate influenza viruses (Table 1).

	Virus, biological sample	Capture efficiency
Human Influenza A	H1N1, culture medium	100%
	H1N1, allantoic fluid	97 %
	H3N2, culture medium	74 %
	H3N2, allantoic fluid	76 %
Human influenza B	B/ Yamagata-like, allantoic fluid	86 %
	B/ Victoria-like, allantoic fluid	78%

Table 1 : Capture efficiency was determined by hemagglutination titer of influenza A viruses (H1N1 and H3N2) and Influenza B (yamagata-like and victoria-like) recovered with Viro-Adembeads from infected culture medium of MDCK cells and from infected allantoic fluid.

Recovery of infectious Human Influenza viruses

Maintaining infectivity after treatment is particularly important because infectious viruses are needed for the development of vaccines. The infectivity of captured virus was determined by using two models (MDCK cells and eggs). The viral concentration was similar to initial virus solution. Polyethylene glycol and ultracentrifugation decrease the infectivity of viruses. There are several methods for concentration using magnetic beads (polyethyleneimine, sulfonate, chitosan). These magnetic particles promote adsorption but no reports showed that the virus is captured without a decrease in infectivity. Taken together, Sakudo and colleague showed that Viro-Adembeads captured efficiently influenza viruses without a decrease in infectivity.

Recovery of infectious Avian Influenza viruses

H5N1 avian influenza viruses is highly pathogenic not only for poultry, but also for humans. Only four strains of avian influenza (H5N1, H7N3, H7N7 and H9N2) are known to cause human infections. The World Health Organisation (WHO) reports that there have been 385 human cases of H5N1 HPAIV infections and 243 deaths as of 19 June 2008.

Dr Sakudo and colleague showed by immunochromatography that highly pathogenic avian influenza viruses H5N1 was also captured by the Viro-Adembeads from allantoic fluid of infected egg and culture medium of primary porcine alveolar epithelial cells. This method is also applicable to others AIV including LPAIV H5N2 and H5N3. Capture efficiency of HPAIV H5N1 was evaluated by ELISA. The quantity of Influenza recovered by Viro-Adembeads was at a similar level that initial virus loading. Finally, the infection rate was evaluated in embryonated eggs. Beads fractions from H5N1 AIV-infected allantoic fluid exhibited a 100 infection rate (similar to initial virus loading). In conclusion, these data demonstrated that Viro-Adembeads efficiently capture infectious avian influenza viruses.