COMPARAISON OF RABIES VIRUS PURIFICATION USING DIFFERENT METHODS

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Rabies is a viral zoonosis caused by negative-stranded RNA viruses of the *Lyssavirus* genus. It can affect all mammals including humans. Dogs are the main source of human rabies deaths, contributing up to 99% of all rabies transmissions to humans. Infection causes tens of thousands of deaths every year, mainly in Asia and Africa. Most of these victims are children under the age of 15. Vaccination against rabies is still the sole way to fight against the disease.

The aim of this work is to compare the purity of rabies vaccine purified by zonal centrifugation and chromatographic methods, in terms of residual DNA level, host cell protein (HCP) level and the overall recovery yield.
For this purpose, Vero cells were grown under animal component free conditions, on Cytodex 1 microcarriers in VP-SFM medium. Vero cell growth and virus production were previously optimized; studies were conducted in a 7-L bioreactor. Virus replication phase was conducted using perfusion culture mode, viral harvests obtained through the culture were clarified, inactivated by BPL (Beta-propiolactone) and then pooled.

The pooled harvests were purified by zonal centrifugation on a sucrose density gradient. The fractions of interest (11 in total) were pooled and checked for their antigenic activity according to the NIH potency test. They showed an activity of 61 UI/ml. The yield obtained was around 60%.

To improve the overall yield, we have tested during a previous work several chromatography matrices (Sephacryl S200, Sephacryl S300, Sepharose 4FF,...). However the yield obtained was not high, around 40% in the best case. To improve this performance, we tested the Capto Core 700 (GE Healthcare Life Sciences) which a matrice that had a dual-functionality: size separation and binding chromatography, and was specially designed for the purification of large biological products such as viruses. The use of this matrice to purify rabies virus resulted in a yield of 84.5%, which was 2-fold and 1.5 fold higher than that obtained using chromatographic columns or zonal centrifugation, respectively.
We also tested Monolytic chromatographic (CIMmultus™ QA-8 Advanced Composite Column) from BIA Separation. Such kind of media represents a new generation of chromatographic matrices with efficient mass transfer and better hydrodynamic properties. This allows fast and efficient separation of large molecules such as DNA and viruses. In our case, we were able to increase the purification yield to values close to 94%. It was the highest yield obtained compared to other methods used.

Currently the content of the purified fractions collected using the different methods is analyzed to estimate the efficiency of DNA and HCP removal.