HIGH DENSITY PERFUSION CELL CULTURES FOR THE PRODUCTION OF MODERN VACCINES AT LOW COST

In November 1984, the World Health Organization and the Rockefeller Foundation initiated a collaborative program to assist key developing countries to produce tissue culture-based rabies vaccines. A laboratory located in Colombia was selected as the first recipient of this program in 1986.

The project wended through many obstacles, but finally in 1999 a license was granted to a company to produce and sell a Vero-based human rabies vaccine in Colombia.

The capacity to produce inexpensive, safe and efficacious vaccines is needed in developing nations to sustain preventive health programs and to gain effective control over the sale and distribution of biological products.

For viral vaccines it implies mastery of large-scale tissue culture production techniques, improved standard operating procedures, newer process controls and standards, and the design and validation of facilities and processes to comply with good manufacturing practices.

The technology developed and in use in Colombia and now in other countries has several unique components: A reduced size vaccine production facility built especially for this purpose with special air handling systems; ultra-high density microcarrier cell cultures adapted to commercial scale fermentors; a especially designed perfusion system to feed growth media for the fermentor and a proprietary decanting column (New Brunswick Scientific), to keep the cells in high concentration and to collect simultaneously high concentration of virus.
Finally a high efficiency system, using column chromatography, to concentrate and purify the vaccine was developed and also a special formulation for a lyophilized product.

One main advantage is the ability to use 25 grams per liter of Microcarrier beads which allows for cell culture densities not previously achieved with other bioreactor, roller-bottles or cell factories. Using these protocols, concentrations of VERO cells of 12- to 14 Million per ml are obtained.

This technology offers numerous advantageous over traditional methods of vaccine production, including a yield 10 times the average quantity obtained by facilities working with roller bottles and about equal to that obtained by facilities using several hundred litter bioreactor run in batch mode with a low concentration of microcarrier beads.

By comparison, those types of traditional manufacturing facilities can require more than a dozen scientists and over a thousand meters of facility space for operation.

Furthermore, when considering the large volume of cell culture media and serum such facilities must use, the benefits of this protocol become even more evident. Unfortunately, most developing nations can not afford such facilities and instead continue to produce vaccines using crudely prepared animal tissue.

Bioreactors are equipped with unique decanting columns that provide high oxygen transfer, high nutrient levels, and the low shear growth environmental that cells require for this level of productivity. The system is designed to allow for continuous perfusion that extends the production cycles of the VERO cells for a period of up to 20 days after viral infection, a considerable improvement over batch cultures.

Additionally, decanting columns prevent the microcarriers with the cells from exiting the bioreactor during perfusion, gently returning them to the vessel and thereby simplifying collection of the budding virus from the spent media.
In addition high density cell cultures are achieved through the use of a special culture media that restricts the acidity and provides a powerful buffer system, making the VERO cells viable for a long period of time.

The new vaccines meet international standards for viral content, quality of antigen, purification level, and immunological capacity and have been tested for consistency, potency and safety in human immunogenicity trials. The vaccine was licensed for use in Colombia, South America at the end of 1999 and now in China at the end of year 2004.

The technology developed could be used for the production of other vaccines of primary importance for the public health community and include diseases such as Influenza, Dengue, Japanese Encephalitis, Polio, Rotavirus, Hepatitis A and several others.

The same technology has been used now to produce a Rabies vaccine for Veterinary use with much less purification but with extraordinary quality and at considerable low prices. This high density cell system is also being used now to produce other veterinary vaccines such as Foot and Mouth Disease and Hog Cholera.

The new Rabies vaccine for human use has other major advantages as compare to similar vaccines on the market. Namely, the new formulation for the final product does not contain any protein from animal origin that could carry unwanted organisms or that could cause reactivity in the patients.

Several projects are being executed to expand the technology to other countries especially to countries in Asia where it is most needed.

SUMMARY

Several countries have for years tested the use of microcarriers for large scale vaccine production without real commercial success. Unfortunately modern industrial vaccine production technology is
not readily available to serve as a model and there are no schools that teach industrial production methodologies.

Developing nations can now adapt this high density microcarrier system to produce many needed vaccines.

This new vaccine methodology has been complemented with a package of purification and formulation procedures, to produce a rabies vaccine that fulfils the international requirements.

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