A COMPLETE CHEMICALLY DEFINED, ANIMAL COMPONENT FREE MEDIUM FOR VERO CELLS AND VIRUS PRODUCTION

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Abstract

Vero cells are a lineage of cells isolated from the kidney of African green monkey by Yasumura and Kawakita in Japan (1962).
Vero cells are used for various purposes, most importantly for the production of cell culture-based viral vaccines. The cell line is among a very limited list of cell lines that have been approved by health authorities for the production of human vaccines.
Vero cells are increasingly used in the production of approved human vaccines protecting against viral infections such as rabies, rotavirus, Japanese Encephalitis and Poliomyelitis. When scale-up is required, Vero cells can be cultured on micro-carrier beads in bioreactors using the appropriate culture medium.
Currently available media contain a vast amount of undefined polypeptides or animal derived sera that may result in batch to batch variations, as well as an increased potential for contamination with adventitious agents and therefore increase the risk for safety issues.
Defined NutriVero™ Flex 10 is optimized for both 2D monolayer and 3D microcarriers suspension cultures, and is suitable for a wide range of a pplications, from large scale cell culturing to virus production. Developed together with Intravacc, an R&D organization for translational vaccinology, this chemically defined, serum-free, animal component-free medium will give you consistent results and maximum control over your virus production process.

Materials and Methods

Cell culture

Vero cells were cultured in NutriVero™ Flex 10, (BI, cat: 05-068-1) as well as a commercially available animal component free (ACF) culture medium in both 2D and 3D culture systems.

2D culture system – Vero cells were seeded in static culture dishes. The cells were detached using Recombinant Trypsin EDTA (BI, cat: 03-079-1), neutralized using Soy Bean Trypsin Inhibitor (BI, cat: 03-048-1) and counted using Viability and Cell Count Assay, Chemometec.

3D culture systems – 3D culture systems included Spinner and Bioreactor cultures, where Cytodex-1 Microcarriers (GE) were used to offer the required growth surface. The bioreactors (Biostat Sartorius) were operated at 2L working volume. Stirring speed was set between 70 and 130 rpm, temperature set to 37ºC and pH controlled to 7.2. The bioreactors were seeded with $0.15x10^9$ cells/L and 3g/L Cytodex-1.

Virus production

Initial assessment of viral production was performed in 2D culture system infected with various viruses. The second phase included a 3D culture system in bioreactors. The virus infection was performed 72hrs post cells seeding. Virus production was assessed by Cytopathic Effect (CPE) monitoring using a light microscopy and virus titers (CCID50).

Soy hydrolysate

0.1% soy hydrolysate (Kerry, cat: Hy Pep 5603N) was added to the medium and the different parameters (cell concentration and virus yield) were measured.

Abbreviations

CD	Chemically Defined	XF	Xeno Free
ACF	Animal Component Free	CPE	Cytopathic Effect
SF	Serum Free		

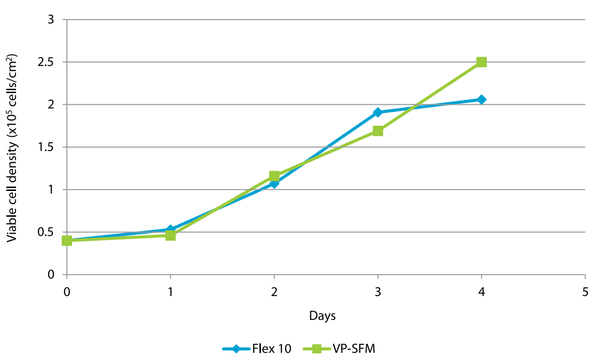
Results

I. Vero Cell Growth

Fig 1:
Vero cell growth and density in 2D and 3D culture systems

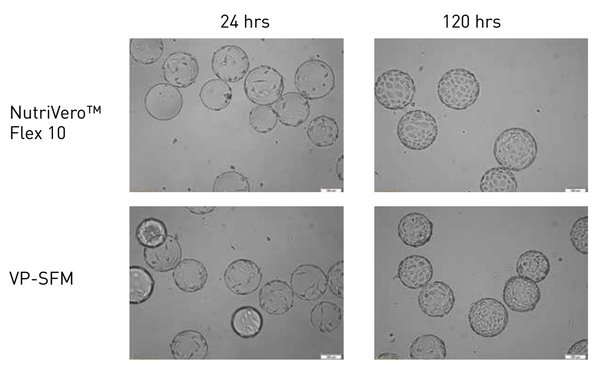
NutriVero™ Flex 10 was tested in a defined ACF system for cell growth and cell density. Utilizing a 2D culture system, NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extract containing medium (VP-SFM).
When tested using Cytodex-1 microcarriers in a 2L bioreactor, Vero cells were adhered to the microcarriers 24hrs following seeding, and after 120hrs all microcarriers were fully confluent with cells homogeneously distributed. NutriVero™ Flex 10 defined medium showed equivalent performance as undefined extract containing medium (VP-SFM).

A.



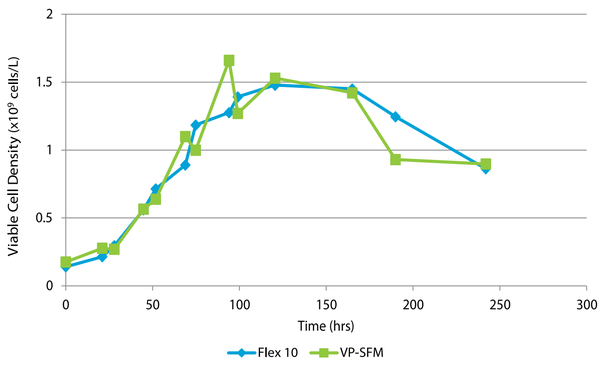
Growth curve of Vero cells in 2D culture system.

B.



Representative images of 3D culture system on Cytodex-1 microcarriers.

C.



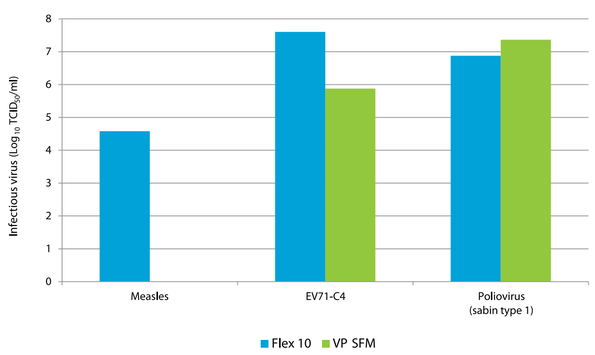
Growth curve of Vero cells in 3D culture system (bioreactor).

II. Virus production

Fig 2:
Virus production in 2D and 3D culture systems

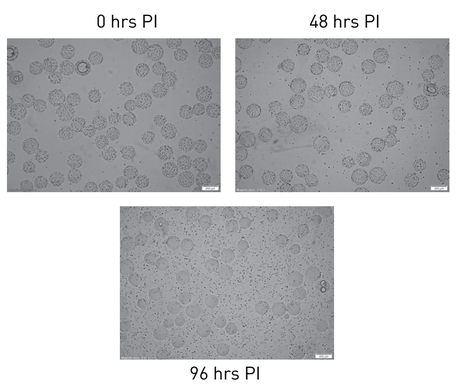
Initial assessment of defined NutriVero™ Flex 10 viral production capacity was performed using a 2D culture system and infection by various viruses. Virus titer for NutriVero™ Flex 10 was comparable to undefined medium (VP-SFM).
For virus production assessment in 3D culture system, Vero cells were cultured in bioreactors and infected with various viruses. Both culture duration, determined by CPE, as well as virus titers using NutriVero™ Flex 10 were found to be better than an undefined control medium (VP-SFM).

A.



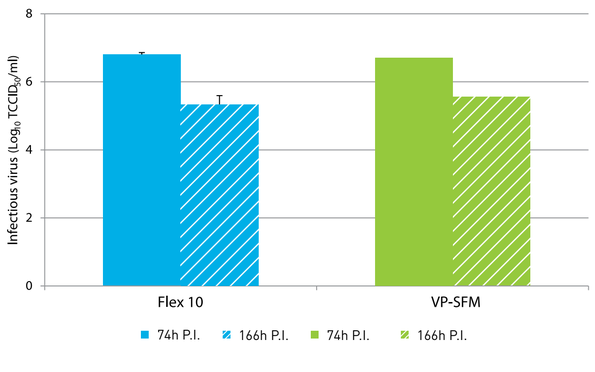
Virus production on Vero cells in 2D culture system.

B.



Representative images of Vero cells grown in 3D culture system and infected with Sabin poliovirus type 3.

C.



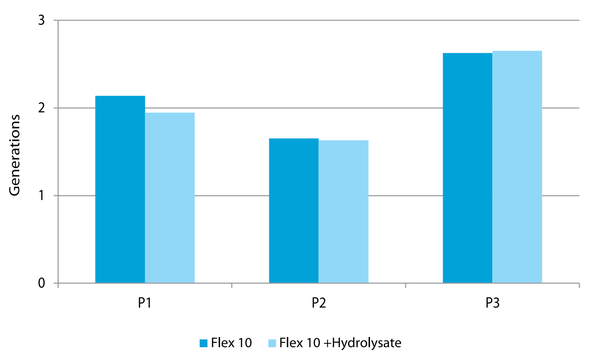
Virus production (EV71-C4) from Vero cells grown in 3D culture system.

III. Soy hydrolysate

Fig 3:
Insignificant effect of soy hydrolysate

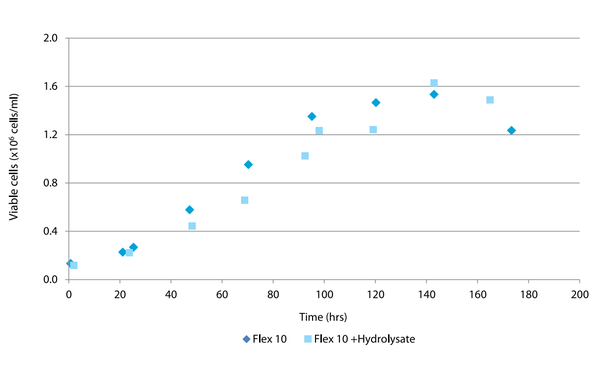
To assess the effect of plant hydrolysate on the performance of defined NutriVero™ Flex 10, 0.1% soy hydrolysate was added to the medium. In both 2D and 3D culture systems the addition of plant hydrolysate had no effect on Vero cell growth.
For virus production assessment, Vero cells were seeded in a 2D culture system and infected with various viruses. The addition of hydrolysate did not significantly enhance the production of any of the viruses.

A.



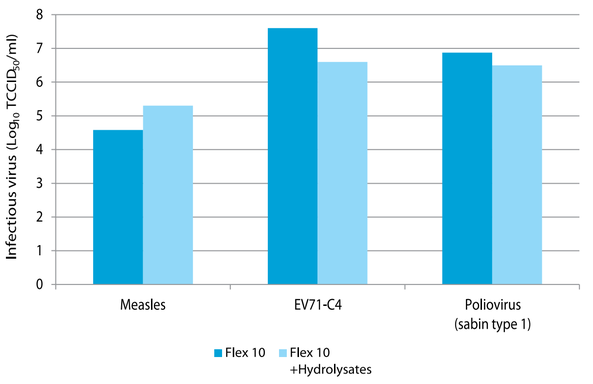
Vero cell concentration was measured during harvesting of 3 passages and the number of generations was calculated. The figure shows the number of generations in each passage. During passage 1 and 3 the cells were grown for 4 days, while during passage 2 the cells were grown for 3 days before harvesting.

B.



Growth curve of Vero cells grown in 3D culture system with or without plant hydrolysate.

C.



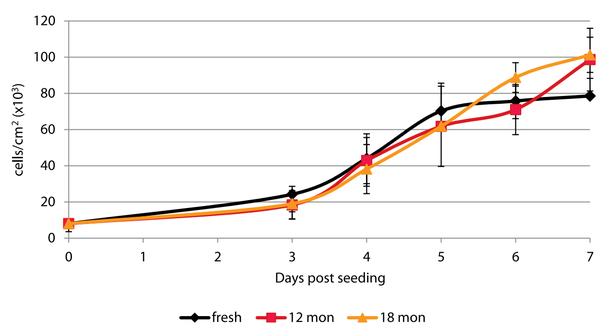
Virus production on Vero cells in 2D culture system with or without plant hydrolysates.

IV. Stability

Fig 4:
NutriVero Flex 10 stability

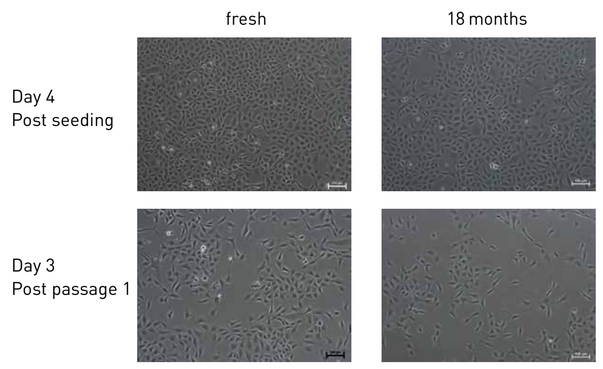
NutriVero™ Flex 10 stability was assessed up to 18 months post production. Utilizing a 2D culture system, Vero cells were seeded in NutriVero™ Flex 10 defined medium and counted at indicated time points.

A.



Vero cells were grown in 2D culture system, harvested and counted.

B.



Images of a representative batch following production and at 18 months post production, in a 2D culture system.

Summary

Chemically defined, serum-free, animal component-free NutriVero™ Flex 10 medium demonstrated excellent results in Vero cell growth and virus yield in both 2D and 3D culture systems.
Containing solely recombinant components and no plant extracts (no hydrolystates) NutriVero™ Flex 10 showed equal performance and in some cases was found to be superior to commercially available undefined medium. Furthermore, the addition of plant hydrolysate to NutriVero™ Flex 10 did not enhance cell growth and virus yield.
Utilizing NutriVero™ Flex 10 removes variability that is in correlation with undefined extracts thus reducing regulatory and health safety concerns as well as manufacturing costs. Its excellent performance proves that utilizing a reliable and safe complete defined system in vaccine manufacturing is now achievable.