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4Cell® Nutrivero™ Flex 10 Medium – Impact of a Chemically Defined Medium on Vero Cells Growth and Productivity Essential in Vaccine Manufacturing

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Introduction

The Vero cell line is now one of the most commonly used cell line for biopharmaceuticals production such as cell culture-based viral vaccines. An example is the successful production of both live (rotavirus, smallpox) and inactivated (poliovirus) viral vaccines[1]. Reasons for the extensive use of the Vero cell line are the consistent high viral yields and relatively easy adaptation for growth in bioreactors on microcarriers, thus allowing greater vaccine purity and as well as quantity[2].

Currently available media to cultivate Vero cells contain undefined plant hydrolysates or animal derived raw materials such as yeastolates. These undefined materials lead to variability in media performance, a result of lot-to-lot variations, increased potential for contamination, as well as inconsistency when scaling-up for commercial processes. Sartorius has introduced 4Cell® NutriVero™ Flex 10 Medium, a chemically defined (CD), serum-free, animal origin–free and hydrolysate-free medium optimized for both 2D monolayer and 3D microcarrier suspension culturing of Vero cells. It reduces process variability and enhancing process safety, providing greater predictability during scale-up and improved virus productivity achieving comparable performance to hydrolysate-containing, serum-free media.

Materials and Methods

Growth Kinetics of Vero Cells in 4Cell® Nutrivero™ Flex 10 Medium

Vero cells were cultured in 4Cell® NutriVero™ Flex 10 (Catalog No. CFV3FA4010) medium as well as a commercially available non animal-origin (NAO) culture media in both 2D and 3D culture systems.
2D culture system – static T-flasks and culture dishes were use and Vero cells were seeded at a cell density of 40,000 cells/cm² and incubated at 37 °C in a humidified controlled atmosphere (5% CO_2).
3D culture system – Cytodex-1 Microcarriers (GE, Catalog No. 17-0448-01) were used in Shake flasks or Bioreactors.
The bioreactors were filled up to 2 L of working volume of tested medium. Stirring speed was set between 70 and 130 rpm, temperature set to 37 °C, pH controlled to 7.2 and the DO controlled to 50% air saturation. The bioreactors were seeded with 0.15 × 10⁹ cells/L and 3 g/L of Cytodex-1 Microcarriers.

Viruses Production Assessment

The initial assessment of viral production was performed in 2D culture system infected with Measles virus, Sabin poliovirus type 1 and Enterovirus 71 (EV71). Next phase included a 3D culture system in bioreactor. Following 72 hours of culture, Vero cells reached a concentration of approximately 1 × 10⁶ cells/mL, EV71 viruses was added. The virus production was assessed by Cytopathic Effect (CPE) using a light microscopy and measuring virus titers.

Results

Vero Cell Growth and Cell Density in 2D and 3D Systems

Vero Cell Growth and Cell Density in 2D and 3D Systems

4Cell® NutriVero™ Flex 10 was tested in a defined animal-component free system for cell growth and cell density. Utilizing a 2D culture system, 4Cell® NutriVero™ Flex 10 chemically defined medium showed equivalent performance as a reference medium containing undefined extracts and non-animal origin (NAO) components (Figure 1).

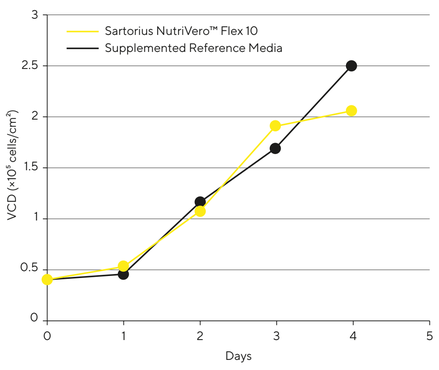

Figure 1: Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm² and incubated at 37 °C in a humidified atmosphere and 5% CO_2 with 4Cell® NutriVero™ Flex 10 or Reference NAO medium.

Vero Cell Growth and Cell Density in a 3D Microcarrier Culture System

4Cell® NutriVero™ Flex 10 was tested using Cytodex-1 microcarriers in a 2 L bioreactor to assess Vero cell growth and cell density under controlled conditions.

Vero cells adhered to the microcarriers 24 hrs following seeding, and after 120 hrs all microcarriers were fully confluent with cells homogeneously distributed (Figure 2A). 4Cell® NutriVero™ Flex 10 medium showed equivalent performance as a reference medium (Figure 2B).

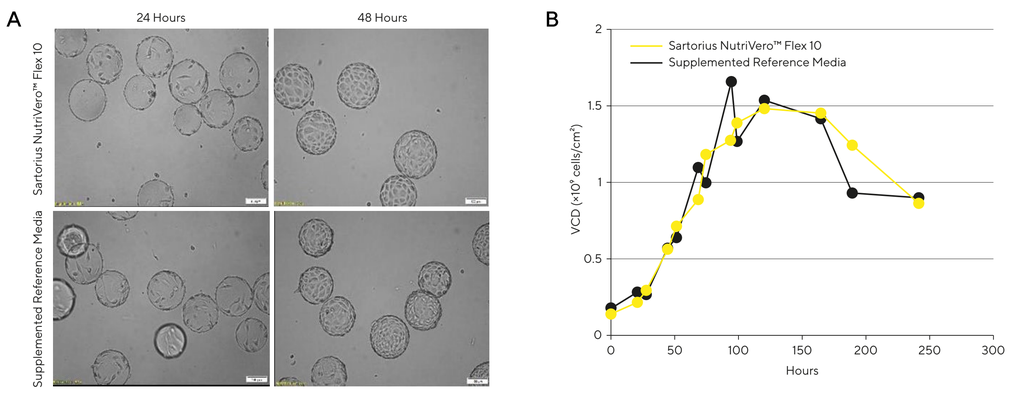

Figure 2: (A) Vero cell growth on microcarriers in 2 L stirred tank bioreactor at 24 and 120 hours. Microphotographs of representative cell culture in defined 4Cell® NutriVero™ Flex 10 and reference medium. (B) Two parallel bioreactors were filled up to 2 L of working volume of defined 4Cell® NutriVero™ Flex 10 and reference medium. Stirring speed was set between 70 and 130 rpm, temperature set to 37 °C, pH controlled to 7.2 and DO controlled to 50% air saturation. The bioreactors were seeded with 0.15 × 10⁹ cells/L and 3g/L of Cytodex-1.

Virus Production in 2D and 3D Systems

Virus Production Assessment in a 2D Culture System

For initial assessment of chemically defined 4Cell® NutriVero™ Flex 10 medium viral production capacity, Vero cells were seeded in a 2D culture system and infected with various viruses (Figure 3A). Virus titer for 4Cell® NutriVero™ Flex 10 was comparable to a as a reference medium containing undefined extracts and non-animal origin (NAO) components.

References

[1] Sheets, R. (2000). History and Characterization of the Vero Cell Line
[2] Yasumura Y., Kawakita Y. A line of cells derived from African green monkey kidney. Nippon Rinsho 21: 1209 — 1210 (1963)

Sabin Poliovirus Type 3 in a 3D Microcarriers Culture System

Chemically defined 4Cell® NutriVero™ Flex 10 Medium sustained cell growth in a 2 L bioreactor and reached a cell concentration of approximately 1 × 10⁶ cells/mL after 72 hrs. All microcarriers appeared homogeneously populated with cells (Figure 3B). Upon reaching such concentrations, Sabin poliovirus type 3 was added to the system and the cytopathic effect was monitored by light microscopy. 4Cell® NutriVero™ Flex 10 Medium did show complete cytopathic effect (>95%) at 96 hrs post infection (PI)

Enterovirus 71-C4 Virus Production in a 3D Microcarriers Culture System

Chemically defined 4Cell® NutriVero™ Flex 10 Medium was tested in a 1 L bioreactor to assess Enterovirus 71 (EV71) production in 3D culture system. Vero cells were cultured in a 1 L bioreactor and infected with EV71 virus (Figure 3C).

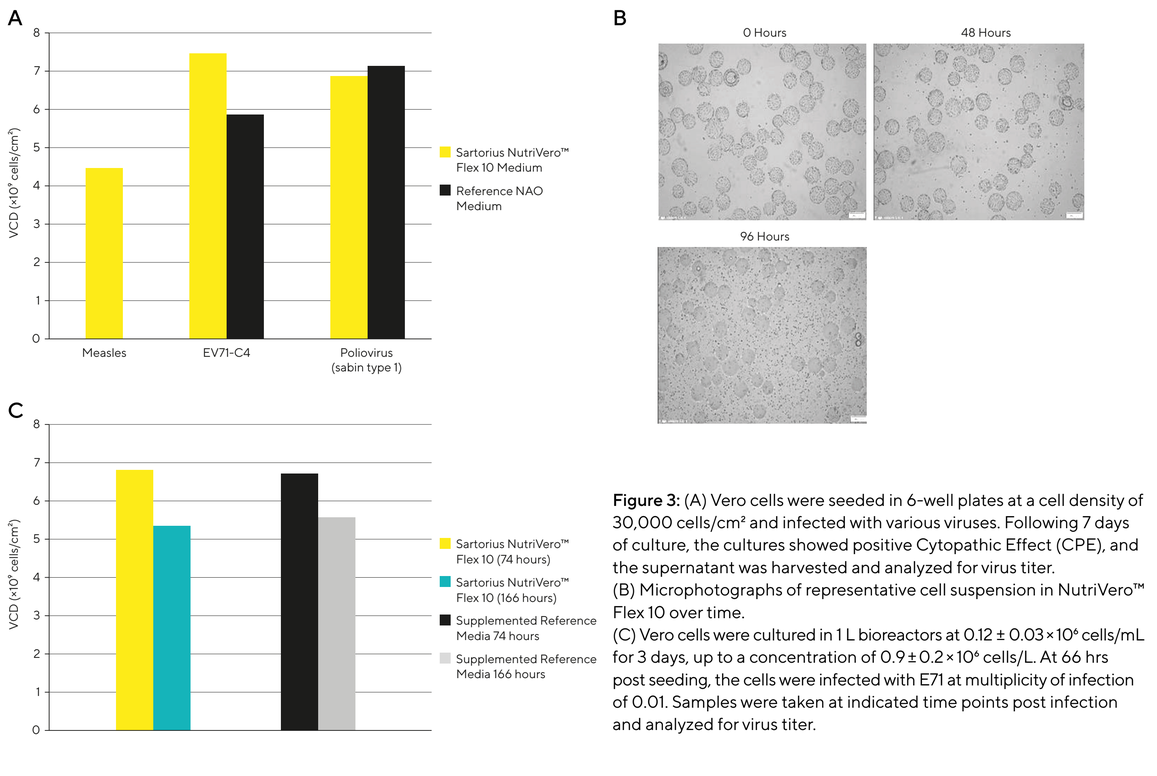

Figure 3: (A) Vero cells were seeded in 6-well plates at a cell density of 30,000 cells/cm² and infected with various viruses. Following 7 days of culture, the cultures showed positive Cytopathic Effect (CPE), and the supernatant was harvested and analyzed for virus titer.
(B) Microphotographs of representative cell suspension in NutriVero™ Flex 10 over time.
(C) Vero cells were cultured in 1 L bioreactors at 0.12 ± 0.03 × 10⁶ cells/mL for 3 days, up to a concentration of 0.9 ± 0.2 × 10⁶ cells/L. At 66 hrs post seeding, the cells were infected with E71 at multiplicity of infection of 0.01. Samples were taken at indicated time points post infection and analyzed for virus titer.

Insignificant Effect of Soy Hydrolysate On Cell Growth and Virus Titers

Vero Cell Growth and Productivity in 2D Culture System With the Addition Of Plant Hydrolysate

To assess the effect of plant hydrolysate on the performance of chemically defined 4Cell® NutriVero™ Flex 10, 0.1% soy hydrolysate was added to the medium.

Vero cells were seeded in a 2D culture system and infected with various viruses: Measles, Sabin poliovirus type 1 and EV71-C4. Following 7 days of culture, all cultures showed positive CPE and the supernatant was analyzed for the amount of infectious virus particles (Figure 4B).

Vero Cell Growth in 3D Culture System With the Addition Of Plant Hydrolysate

The effect of plant hydrolysate was tested in 2 L bioreactor under controlled conditions (Figure 4C). Chemically defined 4Cell® NutriVero™ Flex 10 and 4Cell® NutriVero™ Flex 10 with plant hydrolysate showed similar growth curve.

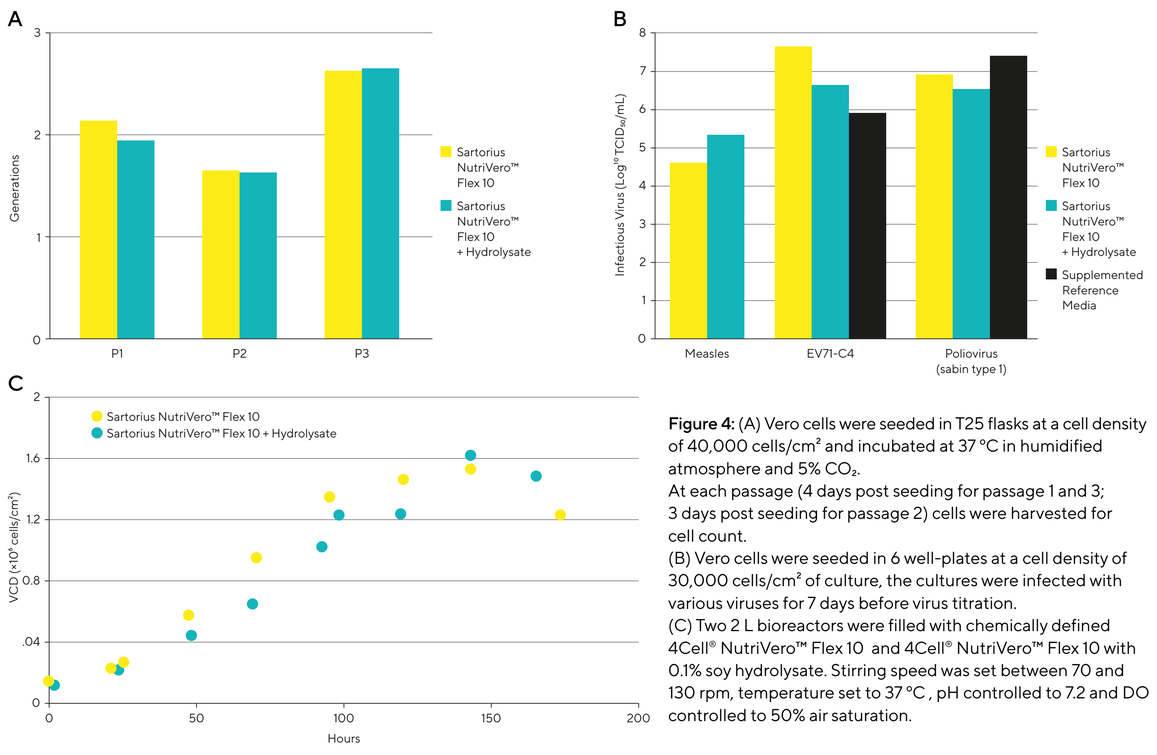

Figure 4: (A) Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm² and incubated at 37 °C in humidified atmosphere and 5% CO_2.
At each passage (4 days post seeding for passage 1 and 3; 3 days post seeding for passage 2) cells were harvested for cell count.
(B) Vero cells were seeded in 6 well-plates at a cell density of 30,000 cells/cm² of culture, the cultures were infected with various viruses for 7 days before virus titration.
(C) Two 2 L bioreactors were filled with chemically defined 4Cell® NutriVero™ Flex 10 and 4Cell® NutriVero™ Flex 10 with 0.1% soy hydrolysate. Stirring speed was set between 70 and 130 rpm, temperature set to 37 °C , pH controlled to 7.2 and DO controlled to 50% air saturation.

Conclusion

The chemically defined 4Cell® NutriVero™ Flex 10 provides the ultimate environment for improved Vero cell viability and yield, as well as high virus production, while maintaining a complete defined animal component-free manufacturing process.

Containing solely recombinant components and no plant extracts (soy hydrolysates) 4Cell® NutriVero™ Flex 10 shows equal performance and in some cases is superior to undefined commercially medium. Furthermore, the addition of plant hydrolysate to 4Cell® NutriVero™ Flex 10 did not enhance cell growth and virus yield. Utilizing 4Cell® 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 proofs that utilizing a reliable and safe complete defined system in vaccine manufacturing is now achievable.