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Application Note

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4Cell® NutriVero™ Flex 10 Medium

Impact of a Chemically Defined Medium for Vero Cells Cultivation and Virus Production for Vaccine Applications

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Abstract

In the race to create serum-free, chemically defined media, research efforts have focused on identifying the elements required for superior growth performance. This include growth factors, hormones, carrier proteins, lipids, transition metals, vitamins, polyamines, and adhesion factors. This application note describes 4Cell® NutriVero™ Flex 10, a new Vero cell medium that is serum-free, chemically defined, and animal component-free. 4Cell® NutriVero™ Flex 10 delivers performance that matches conventional media with undefined hydrolysate supplementation.

4Cell® NutriVero™ Flex 10 has been developed to support Vero cell growth in monolayers and microcarrier suspension culture systems and is optimized for the production of viruses such as measles, Sabin poliovirus Type 1, and enterovirus 71. The Vero cell line is one of a very limited number that have been approved by health authorities worldwide for the production of human vaccines. Its record of safety, quality, and quantity of viral yield is well documented.

1. Introduction

Since the first Vero cell line was isolated in 1962 by Yasumura and Kawakita in Japan [1] from the kidney of the *Chlorocebus sabaeus* (African green monkey), the Vero cell line has become one of the most commonly used for biopharmaceutical production. Examples are the successful production of both live (rotavirus, smallpox) and inactivated (poliovirus) cell culture-based viral vaccines [2]. The extensive use of the Vero cell line is due to consistent high viral yields and relatively easy adaptation for growth in bioreactors on microcarriers, allowing enhanced vaccine quality and quantity [3].

Currently available Vero cell cultivation media contain undefined plant hydrolysates or animal-derived raw materials such as yeastolates. These undefined materials lead to variability in media performance, lot-to-lot variations, increased potential for contamination, and inconsistency when scaling-up for commercial production.

Sartorius has introduced 4Cell® NutriVero™ Flex 10 Medium, a serum-free, chemically defined, animal-origin–free and hydrolysate-free medium optimized for both 2D monolayer and 3D microcarrier suspension Vero cell cultivation. This formulation addresses customer needs and eases pain point by reducing process variability, enhancing safety, providing greater predictability during scale-up and improved virus productivity – achieving performance comparable to serum-free media containing hydrolysates.

2. Materials and Methods

2.1 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 commercially available, non-animal-origin (NAO) culture media in both 2D and 3D culture systems.

The 2D culture system included static T-flasks and culture dishes. Vero cells were seeded at a cell density of 40,000 cells/cm^2 and incubated at 37° C in a humidified, controlled atmosphere (five percent CO_2). Vero cells were detached using Recombinant Trypsin EDTA solution (BI, Catalog No. 03-079-1) or TrypLe Select (Gibco, 12563-29).

The 3D culture system used Cytodex-1 Microcarriers (GE, Catalog No. 17-0448-01) in Shake flasks or bioreactors. The bioreactors were filled up to 2L of working volume of tested medium. Stirring speed was set between 70 and 130 rpm, temperature to 37° C, pH controlled to 7.2, and the DO controlled to 50 percent air saturation. Bioreactors were seeded with 0.15×10^9 cells/L and 3 g/L of Cytodex-1 Microcarriers.

2.2 Virus production assessment

The initial assessment of viral production was performed in a 2D culture system infected with measles virus, Sabin poliovirus Type 1, and Enterovirus 71 (EV71). The next phase included a 3D culture system in a bioreactor. Following 72 hours of culture, Vero cells reached a concentration of approximately 1×10^6 cells/mL, EV71 viruses were added. Virus production was assessed by Cytopathic Effect (CPE) using light microscopy and measuring virus titers.

2.3 Soy hydrolysate

To conduct a peak performance comparison experiment, 4Cell® NutriVero™ Flex 10 medium was used with and without the addition of 0.1% soy hydrolysate (Kerry, Catalog No. HY PEP 5603N). Subsequently, we measured cell concentration and virus yield with and without soy hydrolysate.

3. Results and Discussions

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

3.1.1 Vero cell growth and density in a 2D culture system

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

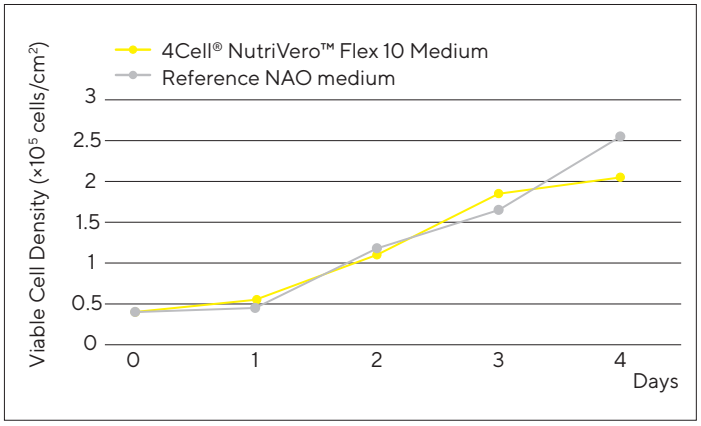

Figure 1: Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm^2 and incubated at 37° C in a humidified atmosphere containing five percent CO_2 using 4Cell® NutriVero™ Flex 10 or a reference non-animal-origin medium.

3.1.2 Vero cell growth and density in a 3D microcarrier culture system

We tested 4Cell® NutriVero™ Flex 10 Medium using Cytodex-1 microcarriers in a 2L bioreactor to assess Vero cell growth and density under controlled conditions. Vero cells adhered to the microcarriers 24 hours following seeding and after 120 hours, all microcarriers were fully confluent with homogeneously distributed cells (Figure 2). 4Cell® NutriVero™ Flex 10 Medium showed equivalent performance compared to a reference medium containing undefined extracts and non-animal-origin (NAO) components (Figure 3).

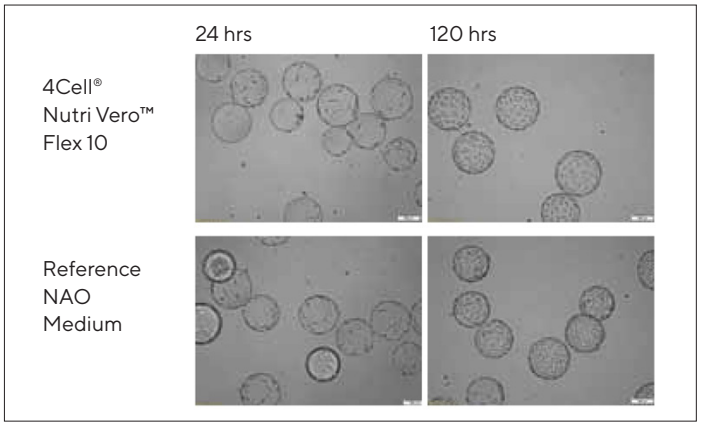

Figure 2: Vero cell growth on microcarriers in 2L stirred tank bioreactor at 24 and 120 hours.

Microphotographs of representative cell culture in 4Cell® NutriVero™ Flex 10 Medium and a reference medium.

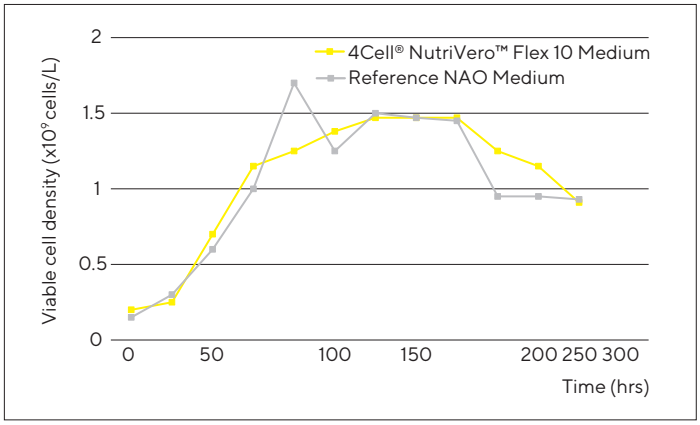

Figure 3: Two parallel bioreactors were filled to 2L of working volume of 4Cell® NutriVero™ Flex 10 Medium and reference medium. Stirring speed was between 70 and 130 rpm, temperature set to 37° C, pH controlled to 7.2, and DO controlled to 50-percent air saturation. The bioreactors were seeded with 0.15 × 10^6 cells/L and 3g/L of Cytodex-1.

3.2 Virus production in 2D and 3D systems

3.2.1 Virus production assessment in a 2D culture system

For the initial assessment of 4Cell® NutriVero™ Flex 10 Medium viral production capacity, Vero cells were seeded in a 2D culture system and infected with several viruses (Figure 4). Resulting 4Cell® NutriVero™ Flex 10 Medium virus titer was comparable to that of a reference medium containing undefined extracts and non-animal-origin (NAO) components.

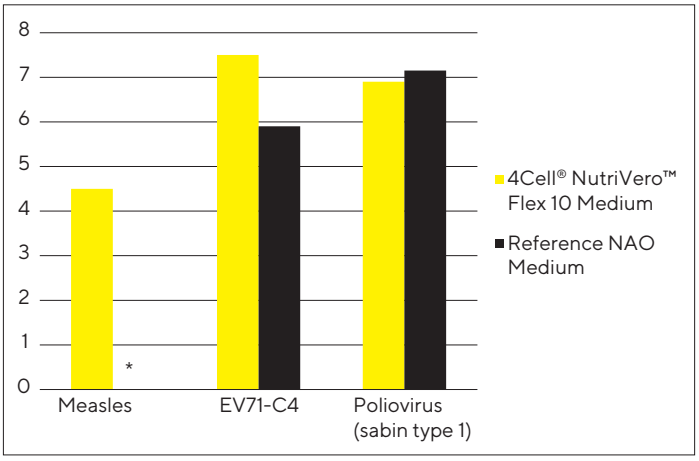

Figure 4: Vero cells were seeded in six-well plates at a cell density of 30,000 cells/cm^2 and infected with several viruses. After seven days, cultures showed positive Cytopathic Effect (CPE), and the supernatant was harvested and analyzed for virus titer.

* Virus titer for measles was below the limit of quantification (LOQ = 4 × $\log_{10}$TCID$_{50}$/ml).

3.2.2 Sabin poliovirus Type 3 in a 3D microcarrier culture system

After 72 hours of culture, 4Cell® NutriVero™ Flex 10 Medium sustained cell growth in a 2L bioreactor and reached a cell concentration of approximately 1 × 10^6 cells/ml. All microcarriers appeared homogeneously populated with cells (Figure 5). On reaching these concentrations, Sabin poliovirus Type 3 was added to the system and the cytopathic effect monitored by light microscopy. 4Cell® NutriVero™ Flex 10 Medium showed complete cytopathic effect (> 95%) at 96 hours post infection, and all microcarriers appeared clear of cells.

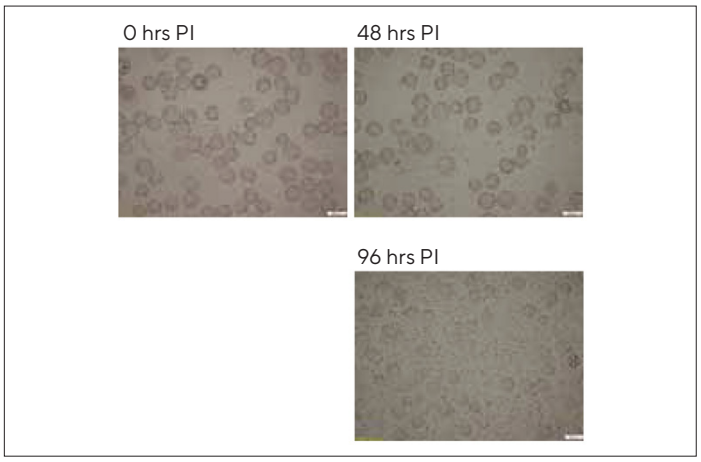

Figure 5: Microphotographs of representative cell suspension in 4Cell® NutriVero™ Flex 10 Medium over time.

3.2.3 Enterovirus 71-C4 virus production in a 3D microcarrier culture system

4Cell® NutriVero™ Flex 10 Medium was tested in a 1L bioreactor to assess Enterovirus 71 (EV71) production in a 3D microcarrier culture system. Vero cells were cultured in a 1L bioreactor and infected with EV71 virus. Virus titer for 4Cell® NutriVero™ Flex 10 Medium was comparable with virus titer produced with an undefined, non-animal-origin reference medium. (Figure

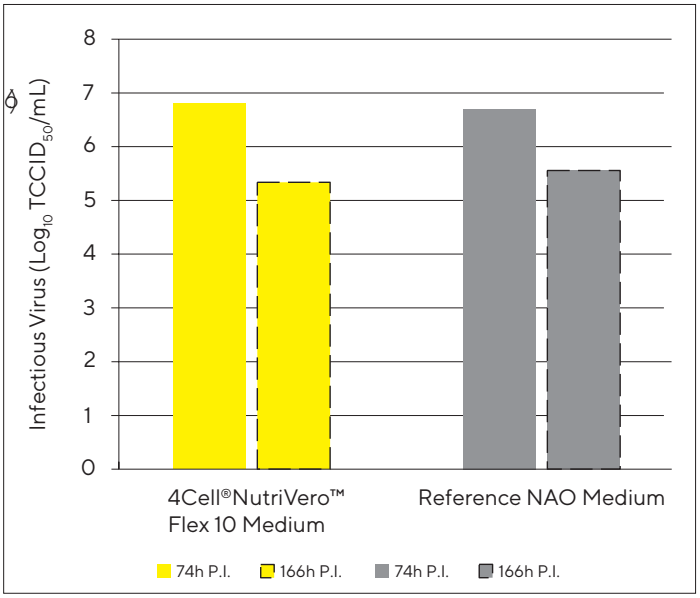

Figure 6: Vero cells were cultured in 1L bioreactors at 0.12 ± 0.03 × 10^6 cells/ml for three days, up to a concentration of 0.9 ± 0.2 × 10^6 cells/ml. At 66 hours post seeding, the cells were infected with E71 at a multiplicity of infection of 0.01. Samples were taken at indicated time points post infection and analyzed for virus titer.

3.3 Insignificant effect of soy hydrolysate on cell growth and virus titer

3.3.1 Vero cell growth in a 2D culture system with added soy hydrolysate

To assess the effect of soy hydrolysate on the performance of 4Cell® NutriVero™ Flex 10 Medium, 0.1% soy hydrolysate was added. Cell concentration was measured on seeding and during harvesting of three passages and the number of generations calculated. We found the addition of soy hydrolysate had no effect on cell growth in a 2D culture system.

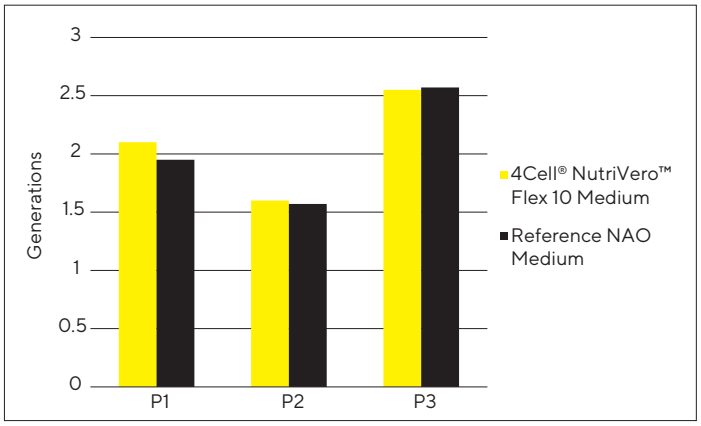

Figure 7: Vero cells were seeded in T25 flasks at a cell density of 40,000 cells/cm^2 and incubated at 37°C in humidified atmosphere with five percent CO_2.
At each passage (four days post seeding for passages one and three; three days post seeding for passage two), cells were harvested and re-suspended in medium for cell count and viability using a nucleocounter N-100.

3.3.2 Virus yield in a 2D culture system with added soy hydrolysate

Vero cells were seeded in a 2D culture system and infected with measles, Sabin poliovirus Type 1, and EV71-C4. Following seven days of culture, all cultures showed positive CPE, and the supernatant was analyzed for the amount of infectious virus particles (Figure 9). We found the addition of soy hydrolysate did not significantly enhance virus production.

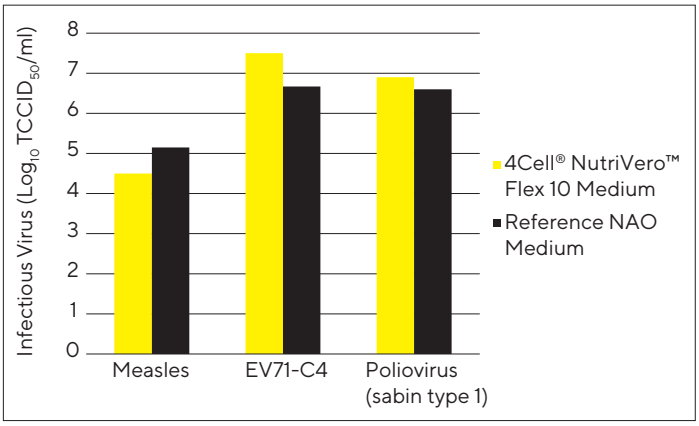

Figure 9: Vero cells were seeded in six-well plates at a cell density of 30,000 cells/cm^2 of culture. The cultures were infected with measles, Sabin poliovirus Type 1, and EV71-C4. After seven days, cultures showed a positive CPE, and the supernatant was harvested and analyzed for the amount of infectious particles via a virus titration procedure.

3.3.3 Vero cell growth in a 3D culture system with the addition of soy hydrolysate

The effect of plant hydrolysate was tested in 2L bioreactor under controlled conditions (Figure 8). 4Cell® NutriVero™ Flex 10 Medium and 4Cell® NutriVero™ Flex 10 with plant hydrolysate showed similar growth curve.

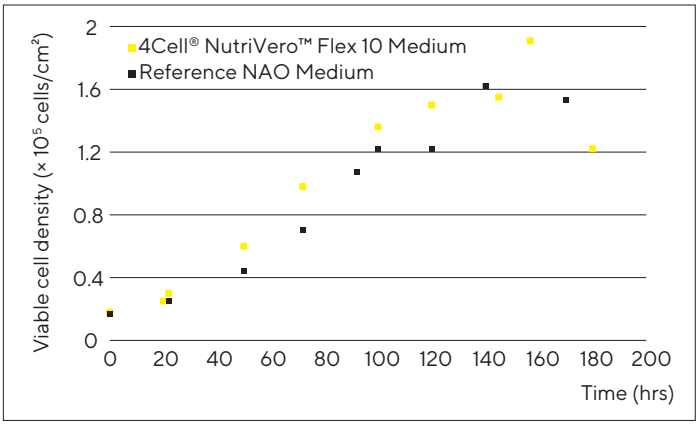

Figure 8: Two 2L bioreactors were filled with 4Cell® NutriVero™ Flex 10 Medium and 4Cell® NutriVero™ Flex 10 Medium with 0.1 % soy hydrolysate added. Stirring speed was between 70 and 130 rpm, temperature set to 37° C, pH controlled to 7.2, and DO controlled to 50 percent air saturation. The bioreactors were seeded with 0.15 × 10^9 cells/L and 3 g/L of Cytodex-1.

4. Conclusion

Cell culture technology has gradually shifted to the use of chemical-based synthetic media because naturally derived ingredients have disadvantages such as large batch-to-batch variation and lower reproducibility. Chemically defined media aims to eliminate inconsistency, simplify downstream processing, and enhance product purity. Sartorius' new, chemically defined, serum-free, animal component-free 4Cell® NutriVero™ Flex 10 Medium produces excellent results for Vero cell growth and virus yield in both 2D and 3D culture systems.

4Cell® NutriVero™ Flex 10 Medium provides the ultimate environment for improved Vero cell viability and yield leading to high virus production while maintaining a chemically defined, animal component-free manufacturing process. Containing only recombinant components and no plant extracts (soy hydrolysates), 4Cell® NutriVero™ Flex 10 Medium demonstrates equal performance and in some cases superior performance compared to an undefined chemical medium. The addition of plant hydrolysate did not enhance cell growth and virus yield. Using 4Cell® Nutri Vero™ Flex 10 Medium removes the variability in correlating with undefined extracts, thereby reducing regulatory and safety concerns as well as manufacturing costs. A safe and reliable chemically defined medium is now available for vaccine production.

5. Bibliography

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