Validation of Cytodex Gamma microcarrier-based virus production in single-use bioreactor systems

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Validation of Cytodex™ Gamma microcarrier-based virus production in single-use bioreactor systems

A majority of cell lines commonly used for viral vaccine production are anchorage-dependent and require expansion on solid surfaces. Here, we describe different approaches for the cultivation of Vero cells using Cytodex and Cytodex Gamma microcarriers in serum-free cultivation medium using spinners, WAVE Bioreactor™ 20/50, ReadyToProcess WAVE™ 25, and Xcellerex™ XDR-10 single-use bioreactor systems. Cells in each WAVE Bioreactor system were infected with influenza virus and the concentration of infectious virus was determined by 50% tissue culture infective dose measurement ($TCID_{50}$). The results show virus titer activity was comparable for Cytodex and Cytodex Gamma and there was no observed difference in distribution or cell growth between systems.

Introduction

Since the 1980s, microcarriers have been used to provide growth support for adherent cells in vaccine biomanufacturing processes at scales up to 6000 L. Today, as yields are increasing, most newly developed vaccines are manufactured at smaller scales, making single-use technologies more applicable. Since anchorage-dependent cell lines grown on microcarriers are mostly sensitive to shear stress, some of the challenges that remain are to have homogeneity and well-suspended microcarriers combined with an efficient oxygenation of the medium.

To demonstrate the utility of disposable bioreactors for the cultivation of adherent cells, Vero cells were adapted and banked in cell culture medium. Optimal conditions were developed for cell cultivation on Cytodex and Cytodex Gamma microcarriers (GE Healthcare) in a WAVE Bioreactor 20/50, ReadyToProcess WAVE 25 bioreactor, and Xcellerex XDR–10 bioreactor* (GE Healthcare). The effects of rocking speed, angle, and working volume on cell growth and virus production were then studied.

*The Xcellerex XDR-10 bioreactor bag for microcarriers incorporated a modified impellor similar to what is found in XDR-50 and XDR-200 bioreactors.

Materials and methods

Cell line and maintenance cultures

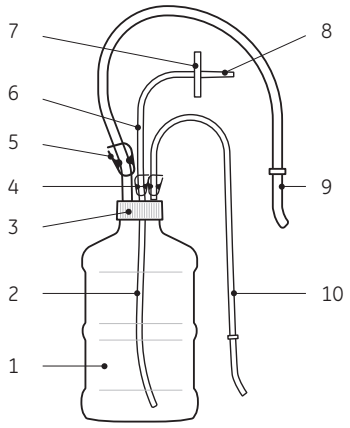
Vero cells derived from African green monkey kidney cells (ECACC) were thawed and cultured in animal-component-free SFM4MegaVir or OptiPRO SFM medium supplemented with 4 mM L-glutamine (Thermo Fisher Scientific) using T-flasks or Cell Factory™ systems (Thermo Fisher Scientific) at 37°C with 5% CO_2. For maintenance cultivation, the Vero cells were washed with PBS prior to addition of recombinant protease for detachment using TrypLE™ Select (Thermo Fisher Scientific) or Accutase™ (GE Healthcare) and incubating for approximately 5 min at 37°C. Cell viability and number were determined in a Cedex™ HiRes Analyzer (Roche) using the trypan blue exclusion method and counting in a Burker chamber using microscopy before inoculation. After detachment from the T-flask, cells were seeded at a density of 4 to 5 × 10^4 cells/cm^2 in a T-flask or Cell Factory system during cell expansion.

Preparation of spinner bottles

Glass spinner bottles were siliconized using Sigmacote™ (Sigma-Aldrich) then washed with distilled water followed by autoclaving for 20 min at 121°C.

Preparation of Cytodex microcarriers

A determined amount of Cytodex microcarriers was weighed in a siliconized glass bottle and PBS was added (50–100 mL/g microcarriers). For swelling, the microcarriers were allowed to stand for 3 h during which they were repeatedly mixed. For sterilization, the microcarriers were washed twice with PBS followed by autoclaving at 121°C for 20 min. The microcarriers were washed twice with cell culture medium (30–50 mL/g) before use.



Item	Part
1	PET container
2	Dip tubing
3	Ported cap
4	Pinch clamp ×2 (small)
5	Pinch clamp (large)
6	Vent tubing
7	Vent filter
8	Protective tubing
9	Transfer tubing (large)
10	Transfer tubing (small)

Fig 1. Cytodex container system with tubing assembly.

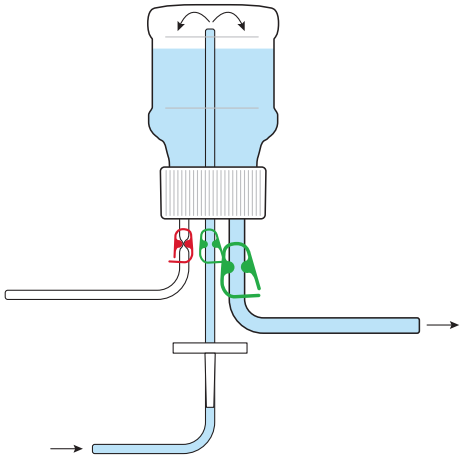


Fig 2. The Cytodex Gamma container system is equipped with a ported cap. Four tubes were fitted to the ported cap: two sealed transfer tubes, one vent tube, and one dip tube connected to the internal port inside the container.

Preparation of Cytodex Gamma microcarriers

Cytodex Gamma microcarriers are supplied ready to use in PET containers (Fig 1) and were therefore transferred directly to the bioreactor using aseptic connections to maintain sterility (Fig 3). The Cytodex container system was mounted upside down with closed pinch clamps and the dip tube positioned above the Cytodex material (Fig 2). The container system was then placed above the bioreactor inlet and pressurized between 0.3 and 0.4 bar with dry sterilized air or inert gas via the vent tubing (air filter tubing) by opening the pinch clamp on the vent tubing for a few seconds. The pinch clamp on the transfer tube was then opened to start the transfer of Cytodex Gamma material into the bioreactor. The air/gas supply on the vent filter was disconnected from the container system once transfer was complete.

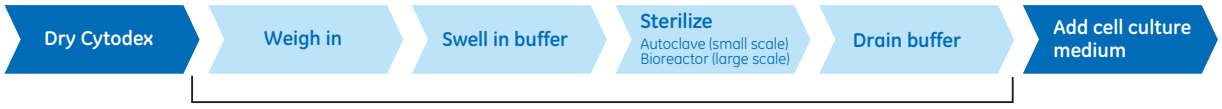
Precoating Cytodex 1 microcarriers

To facilitate cell attachment in serum-free media when cultured in WAVE Bioreactors, Cytodex 1 microcarriers were precoated with Soy Peptone (Sigma-Aldrich) after the last wash and at least 2 h before the cells were added. Cytodex 3 microcarriers did not require pre-coating due to their gelatin layer.

WAVE Bioreactor cultures

A ReadyToProcess WAVE 25 rocker was equipped with two 10 L Cellbag™ bioreactors. One ReadyToProcess™ CBCU controller was used for each bioreactor. Before inoculation, the 10 L Cellbag bioreactors were inflated with air then SFM4MegaVir medium and microcarriers were added. The bioreactors were equalized for at least 2 h at 37°C and 5% CO_2. An offset pH calibration was conducted before

Conventional process:



Simplified process:

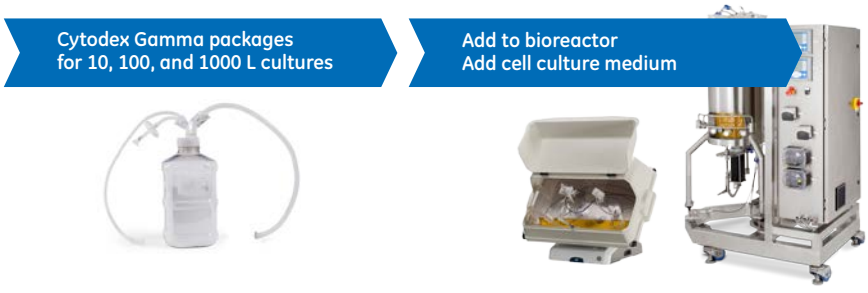


Fig 3. Ready-to-use Cytodex Gamma microcarriers packaged in a single-use container reduce risk by simplifying microcarrier preparation and transfer to single-use bioreactor systems (two bioreactors are shown for illustration only).

inoculation. The Vero cells were washed with PBS prior to addition of recombinant protease for detachment (using TrypLE Select or Accutase and incubating for approximately 5 min at 37°C). Trypsin inhibitor was added and cells were counted. Cells were inoculated at a concentration of 0.2×10^6 cells/mL in a 4 L working volume using SFM4MegaVir supplemented with 4 mM L-glutamine and 0.2% Pluronic F68. The cultures were controlled at pH 7.1, 37°C, and 5% CO_2. Rocking motion was set to either 100% or 30%. At culture initiation, continuous rocking was set to 8 rpm at a 6° angle. When cells reached confluence, the rocking speed was increased to 9 rpm. After 2 h, each bioreactor was sampled to ensure cells had started to attach to the microcarriers. Thereafter, sampling was conducted every 24 h to determine cell density and morphology. Prior to sampling, rocking speed was temporarily increased for 1 min to 20 rpm to ensure a homogenous solution. After 48 or 72 h, 50% of the working volume was exchanged for fresh culture medium to meet the requirements of the increased cell density. When reaching a density of approximately 1×10^6 cells/mL, the cells were infected with virus.

Xcellerex XDR-10 cultures

A benchtop XDR-10 was equipped with 10 L disposable bioreactor bags. Before inoculation, these bioreactors were inflated with air then OptiPRO SFM medium and microcarriers were added. The bioreactors were equalized for at least 2 h at 37°C and 5% CO_2. An offset pH calibration was conducted before inoculation. The Vero cells were washed with PBS prior to addition of recombinant protease for detachment (using TrypLE Select or Accutase and incubating for approximately 5 min at 37°C). Trypsin inhibitor was added and cells were counted using a Vi-Cell™ XR cell counter. The inoculation bottle was thereafter welded to suitable tubing and cells were transferred into the XDR-10 bioreactor bag. Cells were inoculated at a concentration of 0.2×10^6 cells/mL in a 6 L working volume using OptiPRO SFM supplemented with 4 mM L-glutamine and 0.2% Pluronic F68. The cultures were controlled at pH 7.1, 37°C, and 5% CO_2. The DO set point was set to 40% and impeller speed was set to 50 rpm. After 1, 2, 4, 24, 48, and 72 h of cultivation, a sample was collected from the XDR-10 sample connection for determination of cell attachment, cell number, pH, pCO_2, osmolality, and metabolites. Before sampling, agitation was increased to 80 rpm for 5 min. After 48 or 72 h, 50% of the working volume was exchanged for fresh culture medium to meet the requirements of the increased cell density. When reaching a density of approximately 1×10^6 cells/mL, the cells were infected with virus. Material was removed to spinner cultivation for virus propagation (Table 2).

Spinner cultures

The Vero cells were washed with PBS prior to addition of recombinant protease for detachment using TrypLE Select or Accutase and incubating for approximately 5 min at 37°C. Trypsin inhibitor was added and cells were counted then seeded in to siliconized 125 mL spinner bottles at a density of 0.2×10^6 cells/mL in a total volume of 50 mL OptioPRO SFM cell culture medium supplemented with 4 mM L-glutamine and 0.2% Pluronic F68. Cultures were stirred at 40 rpm and sampled for microscopic determination of cell attachment to the microcarriers at 1, 2, and 4 h. Cell counts for determination of cell growth (nucleus counting using Crystal Violet dye [Sigma-Aldrich]) were performed at 24, 48, and 72 h. At 48 h, 50% of the cell culture medium was replaced by fresh medium.

Settings are summarized in Table 1.

Table 1. Bioreactor settings

System	Volume	Rocks per minute (rpm)	Rocking angle (°)
ReadyToProcess WAVE 25	2 L	10	4
ReadyToProcess WAVE 25	4 L	7, 8, and 9*	6
WAVE Bioreactor 20/50	2 L	10	5
XDR-10	6 L	50†	
Spinner bottle	50 mL	40	

*Start rocking at 7 rpm and increase to 8 rpm after 2 to 4 h. Increase to 9 rpm after 2 d.

† Before sampling, increase speed to 80 rpm.

Virus propagation

Influenza A/Salomon Islands/3/2006/IVR145 (H1N1) virus was used for infection. Prior to infection, the amount of virus particles was calculated according to a multiplicity of infection (MOI) specific for the virus strain. Approximately 50% of the medium was discarded from the cell cultures before prepared virus maintenance medium (SFM4MegaVir medium containing trypsin and virus) was added to each bioreactor. The culture temperature was decreased to 33°C. After 1 h, prewarmed medium was added up to the working volume.

On day 4, cells were showing cytopathic effects and the cultures were therefore harvested for analysis of 50% tissue culture infective dose ($TCID_{50}$).

$TCID_{50}$ analysis

The analysis was performed in 96-well microplates with Vero cells grown in SFM4MegaVir or OptiPRO SFM medium. The cytopathic effect in OptiPRO SFM was determined by microscopy 5 days after setup (Table 2).

Results

Culture performance using Cytodex Gamma microcarriers and Cytodex microcarriers in different bioreactors was studied.

WAVE Bioreactor

Comparing the effect of using different volumes of Cytodex in ReadyToProcess WAVE 25 bioreactors, our results show similar cell growth rate and density between Cytodex and Cytodex Gamma cultures (Figs 4 and 5). Vero cells grown on Cytodex Gamma microcarriers were also infected with influenza virus during exponential growth phase at approximately 1 × 10^6 cells/mL (Fig 6). The concentration of infectious virus was determined by $TCID_{50}$ analysis and virus titer activity was comparable for Cytodex and Cytodex Gamma (Table 2).

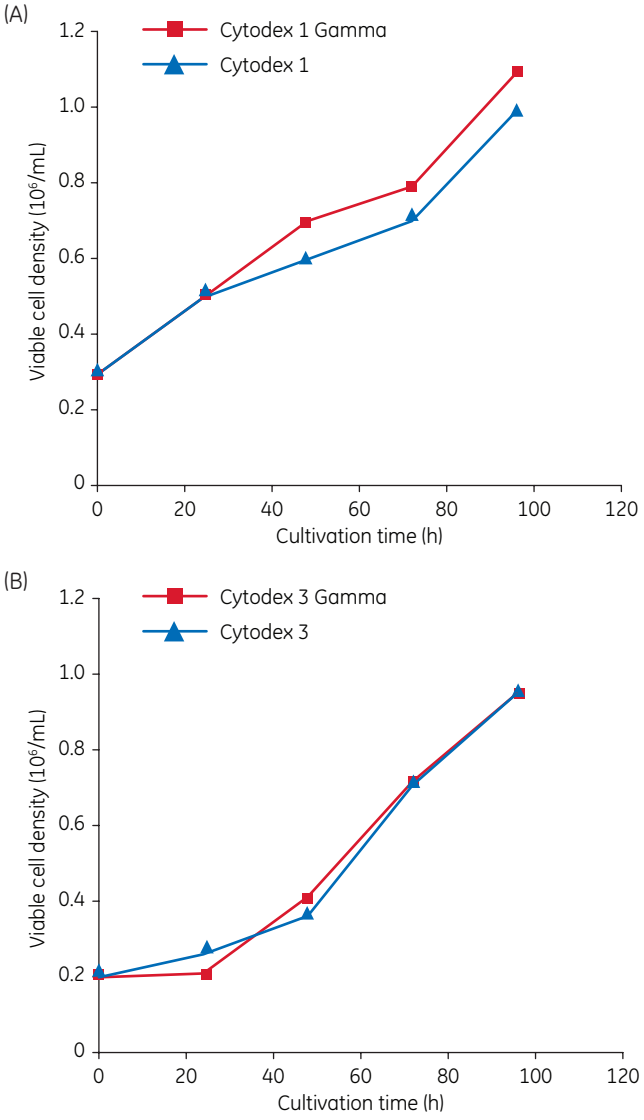


Fig 4. Comparison of cell growth up to 100 h of cultivation between (A) Cytodex 1 and Cytodex 1 Gamma in ReadyToProcess WAVE 25 (2 L), and (B) Cytodex 3 and Cytodex 3 Gamma in ReadyToProcess WAVE 25 (4 L). There was no significant difference in cell growth irrespective of the choice of Cytodex, volume.

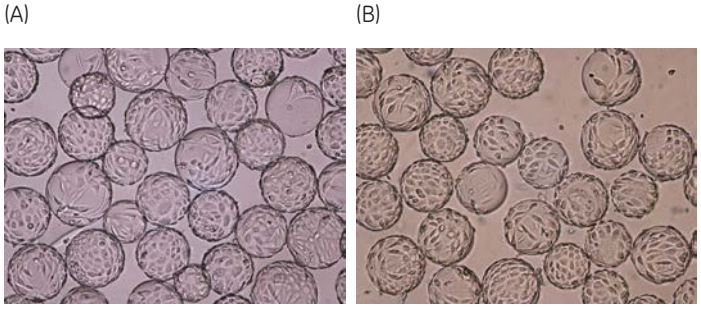


Fig 5. The distribution and cell growth of Vero cells cultured in ReadyToProcess WAVE 25 are similar when grown on (A) Cytodex 1 Gamma and (B) Cytodex 3 Gamma microcarriers for 72 h.

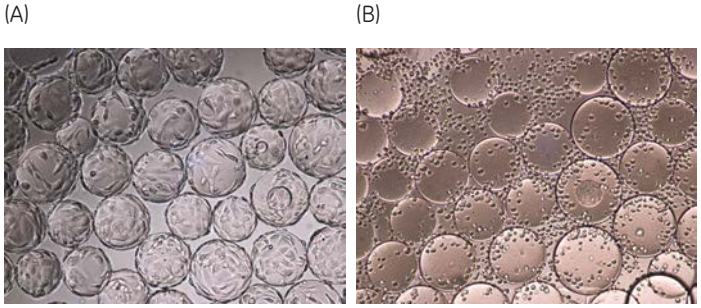


Fig 6. The morphology of Vero cells cultured in ReadyToProcess WAVE 25 and grown on Cytodex 1 Gamma (A) when reaching cell confluence (time of infection) and (B) after 72 h infection (time of harvest) when cytopathic effect was shown.

XDR-10 bioreactor

To determine cell growth on Cytodex and Cytodex Gamma in the XDR-10 microcarrier bag, samples were taken daily after inoculation. Depending on the test, cell growth in the bioreactor bag was followed for up to at least 72 and at most 120 h. The initial cell attachment (1 to 4 h) was very similar in all tests (data not shown) and the highest viable cell count of 1.2 × 10^6 cells/mL was sampled at 72 h (data not shown). As a reference for the attachment, small spinner cultures of 50 mL were run in parallel with the cultivation in the XDR-10 bioreactor. Importantly, cell growth in the XDR-10 bioreactor and control spinner cultures was similar for each individual experiment (Figs 7 and 8).

(A) (B)

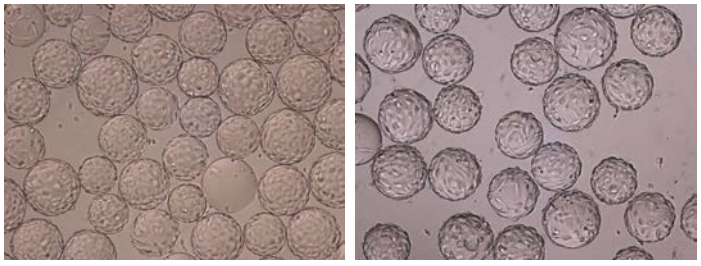


Fig 7. Growth of Vero cells on (A) Cytodex 3 and (B) Cytodex 3 Gamma in an XDR-10 bioreactor 72 h after inoculation.

(A) (B)

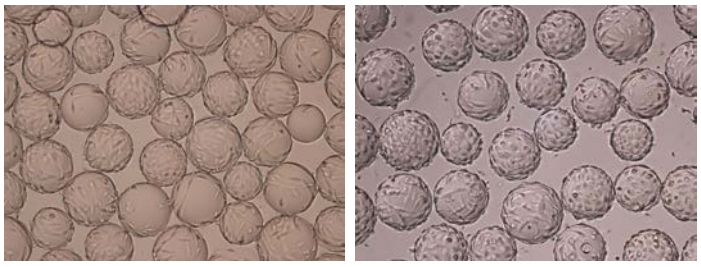


Fig 8. Growth of Vero cells on (A) Cytodex 3 and (B) Cytodex 3 Gamma in spinner bottles 72 h after inoculation.

Figure 9 shows viable cell density in the XDR-10 bioreactor from different experiments. At 48 h cultivation, 2 to 3 L of cell media was replaced by fresh media. After stopping the impeller, the microcarriers were allowed to sediment (5 to 10 min) before cell media was removed through the dip tube.

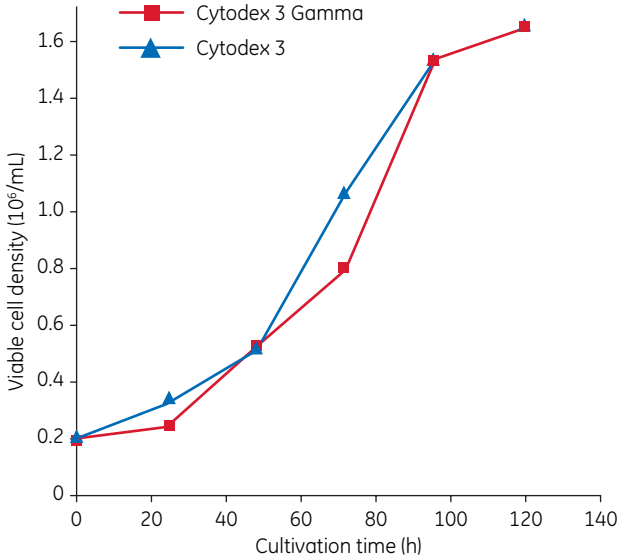


Fig 9. Comparison between Cytodex 3 and Cytodex 3 Gamma in XDR-10 bioreactor (6 L).

To test whether sampling at 50 rpm is representative for a homogenous cell culture, we compared microcarrier pellet size from samples taken at 50 and 80 rpm. Although no obvious differences could be observed between the different agitation speeds, a slightly higher cell number was determined at 80 rpm (data not shown).

Table 2. $TCID_{50}$ analysis of virus propagation in WAVE Bioreactor and XDR-10 cultures

Microcarrier	$TCID_{50}$	Bioreactor system
Cytodex 1	$10^{7.0}$/mL	ReadyToProcess WAVE 25 (2 L)
Cytodex 1 Gamma	$10^{7.3}$/mL	ReadyToProcess WAVE 25 (2 L)
Cytodex 3	$10^{7.1}$/mL	ReadyToProcess WAVE 25 (4 L)
Cytodex 3 Gamma	$10^{7.5}$/mL	ReadyToProcess WAVE 25 (4 L)
Cytodex 3	$10^{7.5}$/mL	Spinner (from XDR-10)*
Cytodex 3 Gamma	$10^{7.4}$/mL	Spinner (from XDR-10)*

*XDR-10 bioreactor was not in a BSL 2 facility so material was removed and the virus propagation performed using a spinner bottle in a BSL2 facility.

Conclusion

Microcarriers need to be maintained in suspension and the culture kept well aerated. Bioreactor design and operating parameters therefore need to be carefully chosen to avoid excessive shear, which can be detrimental for cell growth and virus production. These prerequisites can limit operating conditions such as culture volume and mixing speed under which microcarriers can be used. Our experiments show that by carefully selecting operating parameters, good cell growth and virus yields can be achieved in three different systems. The standard glass bulb stirred system which is considered to be low shear, the single-use XDR-10 marine impeller stirred system which is intermediate shear, and the WAVE Bioreactor mixed single-use system which is high shear due to the collisions of carriers during the turnaround of medium during rocking. In all systems, Cytodex Gamma performance was equivalent to Cytodex regarding both cell growth and virus manufacturing/titer while both XDR-10 and ReadyToProcess WAVE 25 allowed simplified medium exchange, resulting in minimal loss of both microcarriers. A major benefit of using presterilized Cytodex Gamma microcarriers in single-use bioreactors was the removal of time-consuming preparation, sterilization, and cleaning validation processes prior to culture.

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