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# Validation of the production of influenza virus in ReadyToProcess WAVE™ 25 bioreactor system, comparing Cytodex™ and Cytodex Gamma microcarriers

This application note describes the validation of the single-use ReadyToProcess WAVE 25 rocking bioreactor system in production of influenza virus from Vero cells in microcarrier-based cultures. A 2 L process, developed using the predecessor WAVE Bioreactor™ 20/50 system, was used as a starting point. Compared with WAVE Bioreactor 20/50, the successor ReadyToProcess WAVE 25 system allows using a smoother rocking motion to reduce shear. As shear stress is a critical parameter in virus production from anchorage-dependent cells, the possibility to increase working volume to 4 L, without increasing shear forces, was investigated. The results show improved culture performance using a smoother rocking motion. To reduce the need for microcarrier preparation prior to use, presterilized and ready-to-use Cytodex Gamma microcarriers were evaluated and compared with Cytodex microcarriers. The results show comparable culture performance between the Cytodex products.

## Introduction

Cells used for virus propagation are sensitive and easily damaged, for example, by shear stress during culture. Hence, virus production in cell culture is preferably performed in a bioreactor with capacity to aerate increasing cell densities without increasing shear forces.

Compared with its predecessor WAVE Bioreactor 20/50 system, ReadyToProcess WAVE 25 allows different rocking motions to be selected to better control shear stress. In this work, ReadyToProcess WAVE 25 was validated for use in influenza virus production from Vero cells grown on Cytodex microcarriers. The process was developed in previous experiments using the predecessor WAVE Bioreactor 20/50 system. With this system, maximum working volume,

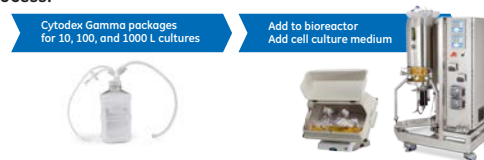
without introducing critical shear stress, was previously shown to be 2 L in a 10 L Cellbag™ bioreactor. Using the ReadyToProcess WAVE 25 system, the possibility of increasing working volume to 4 L by using a smoother rocking motion was investigated.

Vero cells are anchorage-dependent and can only proliferate when provided a suitable surface. In bioreactor cultures, microcarriers are used to meet this requirement. However, Cytodex microcarriers need to be washed, sterilized, and equilibrated before use. For single-use bioreactors, microcarrier preparation is usually conducted in a separate reusable tank. Presterilized Cytodex Gamma microcarriers, on the other hand, can be added directly to the production vessel without prior preparation (Fig 1) reducing the number of unit operations. In the present study, Cytodex Gamma microcarriers were evaluated as an alternative to Cytodex microcarriers. Supplied in containers with flexible connection options for various cell culture vessels, Cytodex Gamma microcarriers not only reduce time and work load but also lower the contamination risk by allowing aseptic transfer from the container to the culture vessel.

### Conventional process:



### Simplified process:



**Fig 1.** Ready-to-use Cytodex Gamma microcarriers packaged in a single-use container simplifies microcarrier preparation and transfer to various single-use bioreactor systems.

## Materials and methods

### Cell line and maintenance cultures

Vero cells (ECACC) were thawed and seeded into serum-free cell culture media in T-flasks before cultured in Cell Factory™ systems. Culturing was performed at 37°C in a 5% CO<sub>2</sub> environment. For maintenance cultivation, cells were washed with phosphate buffered saline (PBS) prior to addition of recombinant protease for detachment using TrypLE Select (Life Technologies). Seeded cell density was approximately 4–5 × 10<sup>4</sup> cells/cm<sup>2</sup>.

Before the cells were added to the bioreactor with microcarriers, trypsin inhibitor was added to the cell suspension and the medium was supplemented with 0.2% poloxamer 188.

### Preparation of Cytodex microcarriers

A determined amount of Cytodex 3 microcarriers was weighed in a siliconized glass bottle and added PBS (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 before being autoclaved at 121°C for 20 min and washed twice with cell culture medium (30–50 mL/g) before use.

### Preparation of Cytodex Gamma microcarriers

The Cellbag bioreactor was inflated with air, after which Cytodex 3 Gamma microcarriers were added by using one of the connectors of its container system, the required amount of Cytodex 3 Gamma microcarriers was transferred directly to the bioreactor under closed system operations, after which serum-free medium was added and the mixture was equalized for at least 2 h at 37°C and 5% CO<sub>2</sub> before cell inoculation.

### Bioreactor cultures

A ReadyToProcess WAVE 25 rocker, connected to one ReadyToProcess™ CBCU controller, was equipped with two 10 L Cellbag bioreactors. Before inoculation, one of the 10 L Cellbag bioreactors was inflated with air and a mixture with serum-free medium and Cytodex 3 microcarriers was transferred to the bioreactor and equalized at 37°C and 5% CO<sub>2</sub>. For the second 10 L Cellbag bioreactor, Cytodex 3 Gamma microcarriers and medium were added as described in Section Preparation of Cytodex Gamma microcarriers. An offset pH calibration was conducted on both culture bags before cell inoculation. Cells were inoculated at a concentration of 0.2 × 10<sup>6</sup> cells/mL in 4 L working volume and cultures were controlled at pH 7.1, 37°C and 5% CO<sub>2</sub>. Rocking motion was set to either 100% or 30%. At culture initiation, rocking speed was set to 8 rpm at a 6° angle and increased to 9 rpm when cells reached confluence. Two

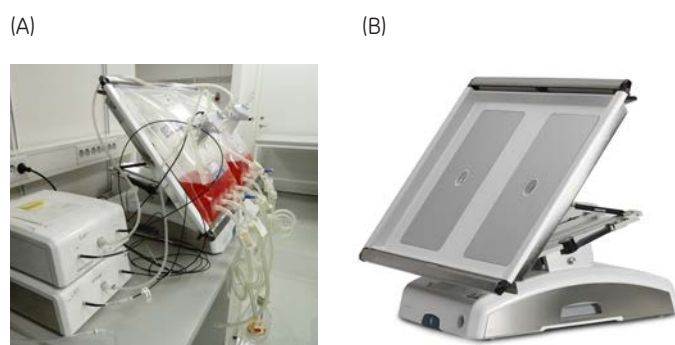
hours after seeding samples were taken to ensure that the cells had started to attach to the microcarriers. Thereafter, sampling was conducted every 24 h to determine cell density and morphology. Prior to sampling, rocking speed was temporarily increased for 1 min to 20 rpm to ensure a homogenous solution. After 48 or 72 h, 50% of the working volume was exchanged for fresh culture medium. When reaching a density of approximately 1 × 10<sup>6</sup> cells/mL, the cells were infected with virus.

### Virus propagation

Influenza A/Solomon 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% medium was discarded from the cell cultures before prepared virus maintenance medium (serum-free medium containing trypsin and virus) was added to each Cellbag bioreactor and the culture temperature was decreased to 33°C. After 1 h, prewarmed medium was added up to the 4 L working volume. The cultures were harvested on day 4 and analysis of 50% tissue culture infective dose (TCID<sub>50</sub>) was performed in 96-well microplates.

## Results

ReadyToProcess WAVE 25 bioreactor system was developed with the goal of keeping the simplicity of single-use rocking bioreactor systems, while enhancing system features with time-saving automation technologies (1). System operation is easily managed via the intuitive and user-friendly UNICORN™ system control software. Operations are facilitated by ergonomic design features such as the tilt function, which in this case, allows easy medium exchange with minimal microcarrier loss (Fig 2). Single-use technology, together with the open configuration, allows for quick start-up and changeover between runs.

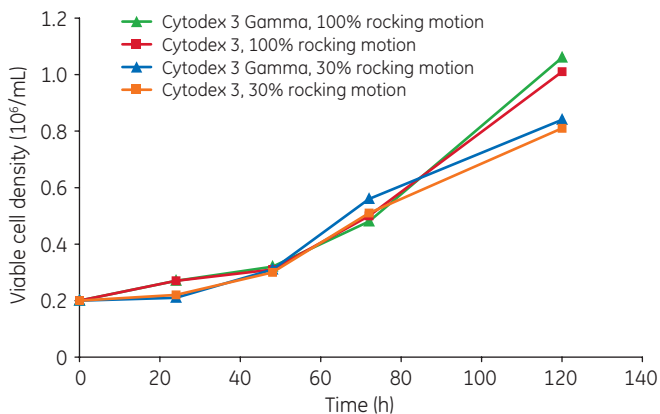


**Fig 2.** (A) The ergonomic design makes activities such as sampling and harvest convenient and easy. (B) ReadyToProcess WAVE 25 in tilt position.

In ReadyToProcess WAVE 25, different rocking motions can be selected to provide suitable conditions for a variety of cell lines, such as robust cell lines with a high oxygen demand or more delicate cells. In this study, the impact of rocking motion parameter setting of 30% and 100% on cell growth and morphology was investigated. While the lower parameter setting (30%) gives a more constant speed, the highest setting (100%) gives a smoother rocking of the culture as the rocking speed is slowed down at the turning points.

In addition, culture performance using Cytodex Gamma microcarriers versus Cytodex microcarriers was compared.

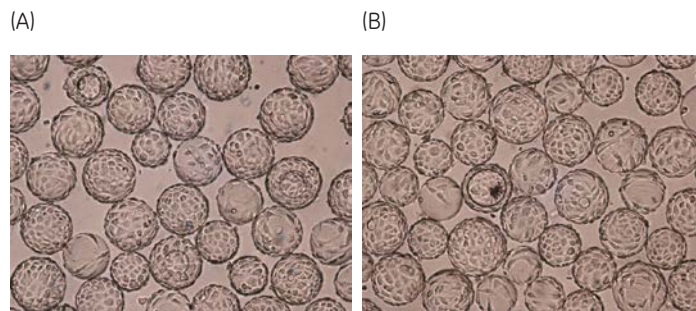
Comparing rocking motions, our results show similar cell growth between 30% and 100% up to 72 h (Fig 3). However, at later time points, as the cell density increased, a smoother rocking motion (100%) was shown to be favorable for cell growth. In Figures 4 to 7, morphology of cells grown on either Cytodex Gamma or Cytodex microcarriers, using either a 30% or 100% rocking motion setting, are shown at time of infection (96 h after inoculation) and at time of harvest (96 h after infection). Obtained results were comparable between cultures using Cytodex Gamma and Cytodex microcarriers. No difference in cell growth or distribution between the cultures could be observed. Also, TCID<sub>50</sub> analysis showed similar results between Cytodex Gamma and Cytodex microcarrier cultures (Table 1).



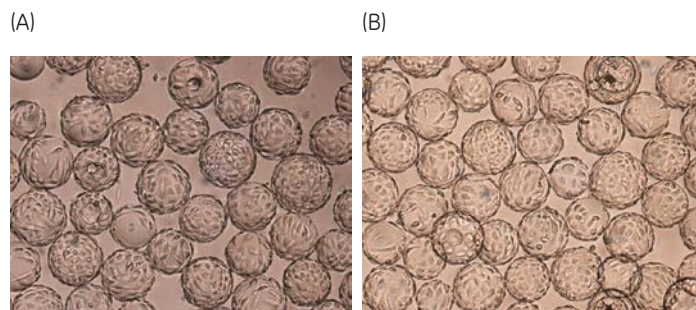
**Fig 3.** Viable cell density in cultures using either Cytodex 3 Gamma or Cytodex 3 microcarriers in 4 L medium. Cells were cultured in ReadyToProcess WAVE 25 using a rocking motion setting of either 30% or 100%.

**Table 1.** TCID<sub>50</sub> analysis results

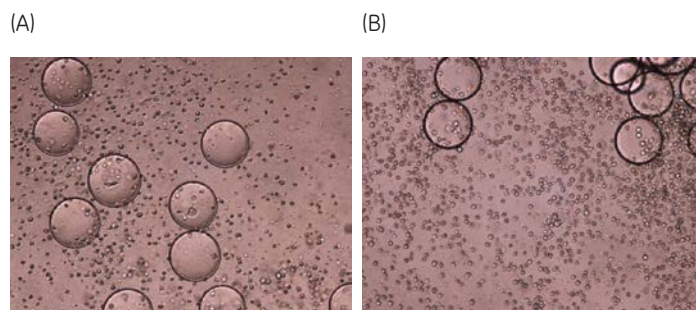
Sample	TCID <sub>50</sub>
Cytodex 3, 100% rocking motion	10 <sup>7.1</sup> /mL
Cytodex 3 Gamma, 100% rocking motion	10 <sup>7.5</sup> /mL
Cytodex 3, 30% rocking motion	10 <sup>7.5</sup> /mL
Cytodex 3 Gamma, 30% rocking motion	10 <sup>7.7</sup> /mL



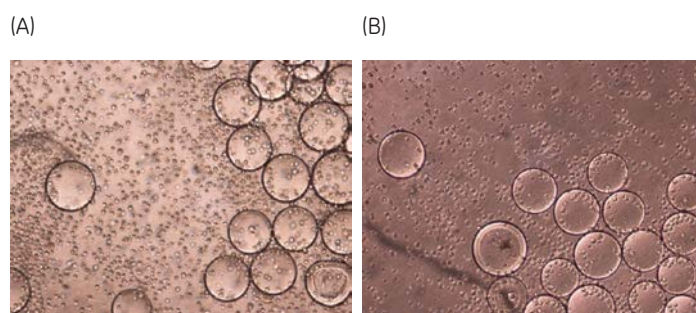
**Fig 4.** Morphology of Vero cells grown on (A) Cytodex 3 Gamma or (B) Cytodex 3 microcarriers 96 h after inoculation. Bioreactor culturing was conducted using a rocking motion setting of 100%.



**Fig 5.** Morphology of Vero cells grown on (A) Cytodex 3 Gamma or (B) Cytodex 3 microcarriers 96 h after inoculation. Bioreactor culturing was conducted using a rocking motion setting of 30%.



**Fig 6.** Cytopathic effect was shown on Vero cells grown on (A) Cytodex 3 Gamma or (B) Cytodex 3 microcarriers 96 h after infection. Bioreactor culturing was conducted using a rocking motion setting of 100%.



**Fig 7.** Cytopathic effect was shown on Vero cells grown on (A) Cytodex 3 Gamma or (B) Cytodex 3 microcarriers 96 h after infection. Bioreactor culturing was conducted using a rocking motion setting of 30%.

## Conclusion

Here, we describe the use of ReadyToProcess WAVE 25 in virus production from Vero cells. The impact of different rocking motions on process outcome was studied. Our results show improved cell growth with a smoother rocking motion (100%) compared with a lower parameter setting (30%). A smoother rocking motion reduces shear, which is critical, for example, for process scale-up. The use of a motion parameter setting of 100% allowed scaling of the process from 2 L to 4 L working volume.

Cytodex Gamma microcarriers and Cytodex microcarriers supported comparable cell growth and virus production. However, presterilized and ready-to-use Cytodex Gamma microcarriers reduce the number unit operation significantly, decreasing time and labor costs.

In conclusion, these results demonstrate a fast and efficient influenza virus production from anchorage-dependent Vero cells grown on ready-to-use Cytodex Gamma microcarriers using the single-use ReadyToProcess WAVE 25 bioreactor system.

## Reference

1. Application note: Significant time savings with simplified cell culturing using ReadyToProcess WAVE 25. GE Healthcare, 29111313, Edition AA (2014).

## Ordering information

Product	Description	Product code
ReadyToProcess WAVE 25	Rocker unit	28988000
ReadyToProcess CBCU	Measurement and controller unit	29044081
ReadyToProcess Pump 25	Pump unit	29032003
Cellbag 10 L	Disposable bioreactor with optical DO and pH sensors	CB0010L10-31
Cytodex 3 Gamma microcarriers	30 g, presterilized	17548801
Cytodex 3 microcarriers	5 kg	17048504

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