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# Allegro™ Microcarrier Delivery System (AMDS)

E-Mail: [Microcarriers@Sartorius.com](mailto:Microcarriers@Sartorius.com)

## 1. Introduction

It has become increasingly evident that closed impeller bioreactors provide an optimal manufacturing solution for vaccines, biologics, and cell therapeutics. The precision controls and small foot print of the reactor coupled with the large surface area for cell culture and growth provided by microcarriers has made these systems a staple in the biotechnology industry where adherent cells are required in large numbers. However, the ease of use of some of the most widely utilized microcarriers has left much to be desired. Some microcarriers available in the market even require pre incubation and several rinses before use. Furthermore, with the advent of single use bioreactors and shift in industry approach towards single-use technologies, traditional approaches to sterilization of microcarriers and their addition to bioreactors need to be revisited. One approach to overcome this hurdle is to use pre-sterilized and ready-to-use microcarriers offered in single-use delivery systems. Eliminating the need for incubating and autoclaving microcarriers prior to use will reduce the preparation time as well as lengthy validations and routine checks of autoclaves and sterilization systems; significantly reducing production times and risks of contaminations, saving pharmaceutical and cellular therapeutic manufacturers effort, time and money.

Herein, we evaluated the Allegro Microcarrier Delivery System (AMDS), a gamma irradiated, ready-to-use microcarrier delivery system to charge single-use bioreactors. The sterility of the microcarriers was evaluated using a sterility test. Functionality of the sterilized carriers was assayed using two different cell types and culture systems. A challenge dose (twice gamma irradiated material) was used to confirm no loss in functionality of irradiated material. Ease of use was evaluated by connecting the AMDS to a standard bioreactor system and evaluating the operators experience with the AMDS and assessing the time it took to set up and deliver carriers into the system.

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## 2. Materials and Methods

### 2.1 Sterilization

AMDS were irradiated at 25 kGy or greater by a partner facility. Briefly, finished goods were loaded into the carrier| tote per established configurations, dosimeters were placed, and the product was exposed to the radiation field (Cobalt 60 source rack). On completion of exposure, dosimeters were analyzed, to confirm that the required dose had been delivered. Twice gamma irradiated material underwent the sterilization process two times. The purpose of twice gamma irradiating material was to demonstrate the robustness of the material against gamma irradiation. Non gamma irradiated materials were autoclaved for 25 minutes at 121 °C in DI water prior to use.

### 2.2 Sterility Test

Sterility testing was performed by directly inoculating the test material (microcarriers dispensed through the delivery system) into Tryptic Soy Broth (TSB) that supports the growth of aerobic and anaerobic bacteria. Test articles were incubated for 14 days (7 days at room temperature and 7 days at 35 °C) followed by testing for microorganism contaminants. The appearance of a cloudy bloom was considered a sterility failure. To evaluate the sterility of gamma irradiated AMDS systems, 50 g of gamma sterilized microcarriers from three irradiated AMDS and one non-irradiated AMDS were cultured in 500 mL TSB for 14 days.

### 2.3 Microcarriers

AMDS were evaluated using the following Sartorius Corporation microcarriers Plastic Plus PP102-1521), Collagen (C-102-1521), Star-Plus (SP102-1521), Fact III (F102-1521).

### 2.4 Vero Cell Culture

For all Vero (American Type Culture Collection (ATCC) CCL-81) cell culture studies, cells at passage 30 or less were used. Complete media, DMEM (Thermo Fisher Scientific SH3058502) containing 5% fetal bovine serum (FBS, Thermo Fisher Scientific SH3007103), 2 mM L-glutamine (Thermo Fisher Scientific SH30034.02), Non-Essential Amino acids (Thermo Fisher Scientific SH3023801) and Antibiotics and Antimycotics (Thermo Fisher Scientific MT-30-004-C1) was used for all stages of culture except for cell seeding of collagen microcarriers (0.05% serum containing media was used). Cell attachment to microcarriers (where applicable) was evaluated at 4 and 24 hours. All cells attached to at least 30 microcarriers in each condition were counted (Power Analysis for ANOVA Design: Power 0.967,  $\alpha$  0.05, Difference of 2, Standard Deviation 2). Cells were seeded, to both culture flasks and microcarriers at  $2 \times 10^4$  cells/cm<sup>2</sup> unless otherwise mentioned. TrypLE\* (Life Technologies, A-12177-01) was

used as the dissociation enzyme, to detach cells from plates and microcarriers (unless otherwise mentioned). Prior to addition of the dissociation enzyme, cells were washed twice with DPBS buffer (Hyclone: Cat# SH3002825). Detachment of cells was performed at 37 °C.

### 2.5 Human Mesenchymal Stem Cell (hMSC) Cell Culture

Bone-marrow-derived hMSCs (Millipore SCR108) were expanded in GIBCO\* low-glucose DMEM (Life Technologies 11054) supplemented with 10% FBS (Thermo Fisher Scientific SH30071.03), 2 mM L-glutamine (Thermo Fisher Scientific SH30034.02), 50 µg/mL penicillin/streptomycin (ATCC 30-2300), and 8 ng/mL basic Fibroblast Growth Factor (bFGF) (EMD Millipore GFO03). "Stem Cell medium" refers to this formulation. To prepare sufficient cells for use in this study, thawed cells were suspended in 10 mL media and pelleted via centrifugation. The medium was decanted, and cells were re-suspended in 10 mL complete medium. Cells were then counted and used to seed T-Flasks at  $5.0 \times 10^3$  cells/cm<sup>2</sup>. Cells were grown for 7 days, with 50% medium exchanges on days 3 and 5, prior to use. Cells were detached from surfaces using same process as for Vero cell culture above. Cells were seeded on microcarriers at  $5.0 \times 10^3$  cells/cm<sup>2</sup>.

### 2.6 Spinner | BioWiggler Culture

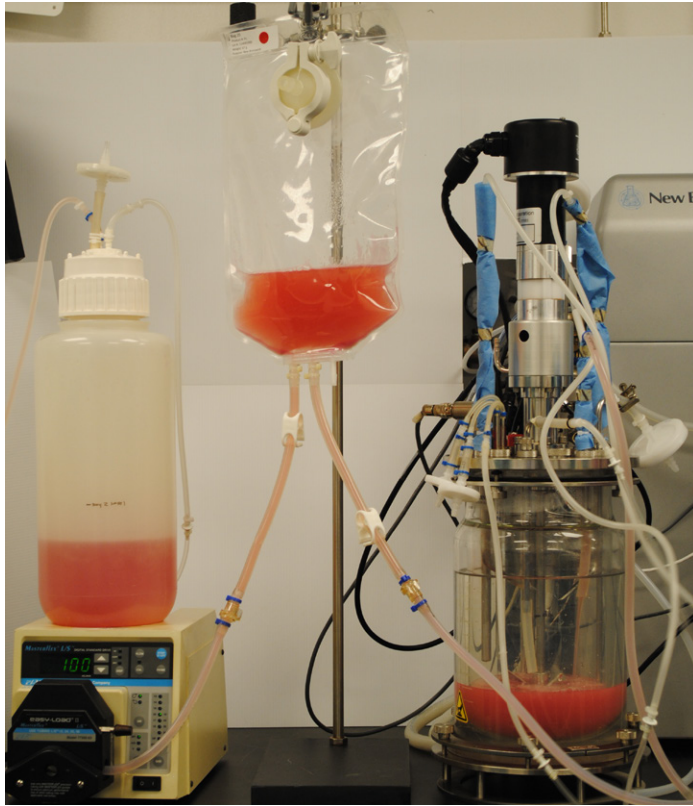
Gamma irradiated microcarriers from AMDS systems were emptied into a sterile container using aseptic technique. A known weight of microcarriers was then transferred to a BioWiggler spinner such that the resultant surface area to volume ratio was 5 cm<sup>2</sup>/mL in a 20 mL volume. Cell culture media, as required for the cell type being cultured, was added to the wiggler and allowed to acclimate for 1 hour. Following acclimation, additional serum was supplemented as needed. Cultures were maintained for 7 days. Cell attachment to microcarriers was assessed at 4 hours and growth was measured on days 1, 3, and 7.

### 2.7 Roller Bottle Culture

For growth studies on microcarriers in a bioreactor, vero cells were cultured and harvested from roller bottles (Fisher 09-761-113). Roller bottles had a surface area that equaled 850 cm<sup>2</sup>. Culture and harvest procedures as per Vero Cell Culture (section 2.4) were used. Roller bottle rotation was set to 0.2 revolution/minute. 200 mLs of complete media was added per roller bottle.

## 2.8 Bioreactor Culture

**Figure 1**  
Bioreactor Set Up



Microcarriers are emptied into the reactor by pumping media to suspend the carriers in the bag, forming a slurry, and subsequently flowing the carriers into the reactor by opening the pinch clamp between the reactor and the AMDS system.

An AMDS filled with 57 g of Star-Plus microcarriers was welded to the input line of the bioreactor (New Brunswick Celligen\* 310 bench top bioreactor). The second tubing line of the AMDS was aseptically connected to 1000 mL of media via a peristaltic pump as in figure 1, using barb fittings. The pinch clamps on the AMDS tubing lines were released, media pumped into the AMDS system and the microcarriers washed into the bioreactor. Detailed procedures for connecting and emptying microcarriers into the bioreactor can be found in the AMDS instructions for use. The entire contents of the AMDS were emptied into the reactor. An additional 800 mL of media was added to the reactor to bring the media volume up to 1800 mL. Vero cells expanded in roller bottles were used to seed the bioreactor culture. Harvested cells were counted and appropriate numbers cells were suspended in 200 mL of complete medium prior to addition to the bioreactor. Once the bioreactor system had equilibrated to the required temperature, pH and DO set points, cells were added to the bioreactor via a peristaltic pump at a constant speed of 50 mL/min.

During this inoculation, an agitation speed of 35 rpm was maintained to facilitate attachment. The temperature, dissolved oxygen (DO) concentration and pH were maintained at 37 °C, above 30% air saturation, 7.35 pH respectively. For the duration of the culture, gas overlay and sparging were used to maintain pH and DO at the desired set points. The bioreactor culture was maintained for 7 days and cell culture growth was assessed on days 1, 3, 5, 6 and 7 as per procedures described in section 2.9.

## 2.9 Cell Counts

Microcarrier culture growth samples were obtained from spinners using a pipette, or from the bioreactor using a sterile syringe sampling assembly. Prior to sampling from the bioreactor, the sample line was purged to remove any non-representative culture from the line from previous samplings, and a fresh 5 mL sample was obtained to evaluate cell density on microcarriers. To obtain counts, cells were enzymatically harvested off the microcarriers and counted using a NucleoCounter\* NC-200 automated cell counter (ChemoMetec-AJS) as per the instructions for use. Briefly, a sample from a spinner or the syringe sample line was transferred into a 15 mL conical tube and the microcarriers were allowed to settle by gravity. A known volume of media was removed from the sample tube and rinsed with PBS solution 2 times. Trypsin was used to harvest the cells. Porcine trypsin at a working solution of 0.05 % was prepared from 0.25% porcine trypsin (Life Technologies: Cat# 15050-065) by diluting in Dulbecco's phosphate buffer saline (DPBS) (Hyclone: Cat# SH3002825) containing 0.5 mM EDTA. Following the two PBS washes, enzyme was added at 0.026 mL per cm<sup>2</sup> (sufficient to cover the microcarriers) and was transferred to a 37 °C humidified incubator for 3-4 minutes to facilitate cell detachment. After 37 °C incubation, cells were examined for cell rounding and detachment from the microcarriers. The trypsinization process was continued for 2 more minutes at room temperature if the cell detachment process appeared incomplete. Tubes were gently tapped on the sides to dislodge rounded cells and cells were washed off with a known amount of media. Once the microcarriers settled, the cell suspension was pipetted into a new tube. A second rinse of the microcarriers with media was performed to ensure complete cell recovery. The resulting cell suspension was used for cell counts. Cell counts obtained using the NucleoCounter NC-200 were verified by manual counting with hemocytometer. All counts were normalized for the weight of the microcarriers from which they were harvested.

\*CelliGen is a trademark of New Brunswick Scientific, BioWiggler is a trademark of Global Cell Solutions, Gibco and TrypLE are trademarks of Life Technologies, and NucleoCounter is a trademark of ChemoMetec AJS.

### 3. Results

#### 3.1 25 kGy Gamma Irradiation Sufficient to Sterilize SoloHill® Microcarriers

To evaluate the effect of gamma irradiation on AMDS systems 50 g of gamma irradiated microcarriers from three AMDS and one non-irradiated AMDS were inoculated into 500 mL of TSB in a sterile jar. The TSB medium and microcarriers

were maintained at room temperature for 7 days followed by 7 day incubation at 32-35 °C. Only the non-irradiated material presented a bloom that was visible. No bloom was noted in any of the irradiated material conditions at day 14.

**Table 1**

Sterility test results demonstrate that 25 kGy is sufficient to sterilize microcarriers manufactured by SoloHill®.

AMDS Sample No.	Gamma Irradiated	Microcarrier Type	Pass	Fail
1	At least 25 kGy	Collagen	■	
2	At least 25 kGy	FACT	■	
3	At least 25 kGy	Star-Plus (SP)	■	
4	No (Not Autoclaved)	Star-Plus (SP)		■

#### 3.2 Gamma Irradiation Does Not Impact Functionality of Microcarriers

The impact of gamma irradiation on the function of microcarriers was assayed in BioWiggler dynamic cultures. Gamma irradiated, twice gamma irradiated and autoclaved Star-Plus microcarriers were cultured with Vero cells for 7 days.

The cell attachment to the microcarriers at 4 and 24 hours post-incubation were assayed for the three different conditions. Total cell counts were assayed at 72-96 hours and 168-192 hours. Cells were seeded at  $2 \times 10^4$  cells/cm<sup>2</sup>.

**Table 2**

Gamma irradiation (up to a total of 50 kGy, 2 x 25 kGy) does not impact Vero cell attachment or growth of cells on SoloHill® Star-Plus microcarriers (A-Autoclaved, γ- Gamma Irradiated, γγ- twice Gamma irradiated). Average counts per microcarrier with standard deviations reported for cell attachment.

Sterilization	Experiment	Cells/Microcarrier		Total Cells/mL	
		4 hr	24 hrs	72-96 hrs	168-192 hrs
A	1	15.9 ± 2.9	18.5 ± 7.8	1.39E+05	8.93E+05
γ	1	15.3 ± 2.5	18.7 ± 4.0	1.28E+05	6.03E+05
A	1	15.9 ± 4.0	18.9 ± 4.5	0.88E+05	7.27E+05
A	2	12.4 ± 3.6	11.2 ± 3.6	3.35E+05	5.17E+05
γγ	2	11.4 ± 3.1	13.6 ± 5.5	0.81E+05	2.45E+05
γγ	3	14.8 ± 8.3	33.6 ± 14.3	3.72E+05	7.39E+05
A	3	21.4 ± 10.5	28.5 ± 10.9	4.28E+05	8.01E+05

**Table 3**

Gamma irradiation of microcarriers (up to a total of 50 kGy, 2 x 25 kGy) does not impact hMSC attachment or growth on SoloHill® suite of microcarriers. Average counts with standard deviations reported. (A-Autoclaved, γ- Gamma Irradiated, γγ- twice Gamma irradiated).

Sterilization	Type	24 hour Cells/ microcarrier	72 hour Cells/ microcarrier
Autoclave	Collagen	3.3 ± 1.8	5.5 ± 3.2
γ	Collagen	3.2 ± 2.3	4.1 ± 1.8
γγ	Collagen	3.1 ± 1.7	4.5 ± 2.3
Autoclave	FACT III	4.4 ± 2.0	4.9 ± 2.5
γ	FACT III	2.8 ± 1.9	4 ± 1.9
γγ	FACT III	3.3 ± 1.8	6.3 ± 2.3
Autoclave	Plastic Plus	3.2 ± 2.3	2.6 ± 2
γ	Plastic Plus	3.1 ± 1.7	3 ± 2.3
γγ	Plastic Plus	4.4 ± 2	2.2 ± 1.7

No statistical differences in cell attachment were noted across sterilization conditions at 4, 24 72 or 168 hours (ANOVA: α: 0.05, P-Value: 0.631, 0.157, 0.359, and 0.254 respectively, with Sterilization method as single factor). Additional testing on the impact of gamma irradiation on microcarriers was conducted with Collagen, FACT and Plastic Plus microcarriers using hMSC cells. For this evaluation hMSCs were cultured on gamma irradiated, twice gamma irradiated and autoclaved microcarriers for 3 days and the attachment efficiency determined at 24 hours and 72 hours. Cells were seeded at  $5.0 \pm 0.2 \times 10^3$  cells/cm<sup>2</sup> and cultured with stem cell media.

No Statistical differences were noted across sterilization modes or microcarrier type with hMSC cultures. (ANOVA α: 0.05, P-Value = 0.270 and 0.667 for Day 1 and 0.285 and 0.254 for Day 3, Sterilization and Microcarrier Type respectively).

## 4. Conclusions

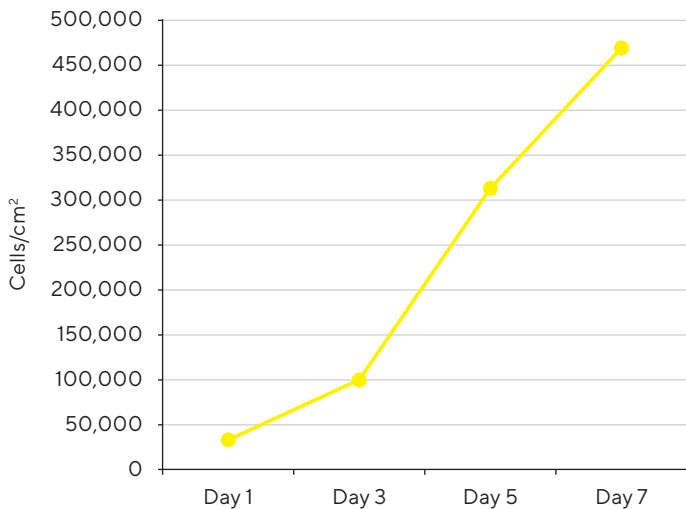
The results presented here demonstrate that the Allegro Microcarrier Delivery System can be used to conveniently deliver gamma sterilized microcarriers into a bioreactor. Gamma sterilization does not impact the functionality of Collagen, Plastic Plus, FACT III or Star-Plus microcarriers offered by Sartorius Corporation. Twice gamma irradiated material was used as a challenge dose to confirm full functionality in microcarriers post irradiation. The second gamma dose is not required for sterilization. There was statistically no loss in function in any of the gamma irradiated microcarriers tested. Additional, testing on the Hillex<sup>®</sup> and Plastic microcarriers packaged in AMDS have shown similar results (results not included in this study). The AMDS system provides a convenient and easy approach to charge bioreactors with microcarriers without the additional time and effort required for washing or sterilizing microcarriers prior to use. Using the AMDS we were able to reduce our bioreactor set up time by approximately 3 hours (20%).

### 3.3 AMDS Provides an Easy and Ready to Use Format for Microcarrier Delivery into Bioreactors and Scale Up.

To evaluate the ease of use of the AMDS system, it was connected to a New Brunswick Celligen 310 bioreactor for a 7 day Vero cell culture. Two connections were required to connect the AMDS system to the bioreactor. A ¼ inch (internal diameter) ID barbed fitting was attached between the bioreactor and the AMDS and ¼ inch ID weld was made between the AMDS and a complete media source (Figure 1). 250 mL of medium was pumped into the AMDS and allowed to form a media-microcarrier slurry in the bag. Once homogenous, gravity was sufficient to empty the microcarriers into the bioreactor. A second rinse of the AMDS with an additional 250 mL of medium emptied all visible microcarriers in the AMDS into the bioreactor. The entire process, from attachment of the AMDS to discharge of the microcarriers into the bioreactor was completed in less than 30 minutes. 57 g of microcarriers was discharged into the system. Total media added to the reactor was 2000 mL. The resulting microcarrier concentration was 10 cm<sup>2</sup>/mL. No washing or additional rinses of microcarriers were required prior to cell seeding or culture. Cell seeding of the bioreactor was performed as previously described, through a dedicated tubing line on the bioreactor. Cells were seeded at 2 x 10<sup>4</sup> cells/cm<sup>2</sup> and the bioreactor culture was maintained for 7 days. Resulting cell counts are summarized in Figure 2.

**Figure 2**

Vero cell growth over seven days in a bioreactor using gamma irradiated Star-Plus Microcarriers




**Germany**

Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen  
Phone +49 551 308 0

**USA**

Sartorius Stedim North America Inc.  
565 Johnson Avenue  
Bohemia, NY 11716  
Toll-Free +1 800 368 7178

 For further contacts, visit  
[www.sartorius.com](http://www.sartorius.com)