

Technical Bulletin

Long-Term Stability of Liquid Cell Culture Media in Bioprocess Bags Composed of EVA (Ethyl Vinyl Acetate) and PE (Polyethylene) Films

Introduction

As an industry leader in the production of cell culture media, SAFC Biosciences considers the quality, sterility and stability of our media to be of paramount importance to our customers. There is an increasing demand in the pharmaceutical and biological industries for large-scale, disposable and flexible liquid media packaging systems. SAFC Biosciences has responded to this demand by developing a variety of bioprocess bags for bulk packaging of liquid media. SAFC Biosciences offers two types of bioprocess bags produced with either an Ethyl Vinyl Acetate (EVA) or Polyethylene (PE) fluid contact surface.

To optimize these bags for packaging of liquid cell culture media, SAFC Biosciences has rigorously investigated the physical properties of the bags and tested the bags for chemical and biological compatibility. Both film types: provide a barrier to oxygen, nitrogen and carbon dioxide; are chlorine free; are stable when gamma irradiated; are translucent; are puncture resistant; are non-leaching and inert; and meet the criteria for USP Class VI Medical Grade Plastic.

The current study was designed to evaluate the stability of liquid cell culture media in EVA and PE bags from two manufacturers (SAFC Biosciences and Stedim). The bags were aseptically filled with Dulbecco's Modified Eagle's Medium (DMEM) and were stored under controlled conditions (2 to 8 C) for a period of 60 weeks. At eight time points during the study, the media within the bags were examined for visual changes including color change, turbidity or precipitation and pH. In addition, an evaluation of growth promotion was performed at the initiation and conclusion of the study.

This study concluded that the liquid cell culture media stored in both SAFC Biosciences' and Stedim EVA and SAFC Biosciences' PE bioprocess bags remained stable with no visible deterioration or change in pH and retained growth promoting properties over 60 weeks.

Materials

Liquid Media for Bags

- Dulbecco's Modified Eagle's Medium (DMEM/High Modified), SAFC Biosciences, Catalog No. 51444

Control Media in PETG (Polyethylene Terephthalate Glycol) Bottles

- Dulbecco's Modified Eagle's Medium (DMEM/High Modified), SAFC Biosciences, Catalog No. 51444

Cells

- Vero, American Type Culture Collection, ATCC No. CCL-81

Bioprocess Bags

- 10 L SAFC Biosciences' Empty PE Bag, SAFC Biosciences
- 10 L SAFC Biosciences' Empty EVA Bag, SAFC Biosciences
- 10 L Flexboy®, Stedim
- 10 L Stedim 5, Stedim

Note: The Stedim Flexboy and the Stedim 5 are proprietary films that have an EVA contact surface

Other

- Trypsin - EDTA (1X Solution) (0.25% Trypsin, 0.1% EDTA), SAFC Biosciences, Catalog No. 59429
- L-Glutamine Solution (200 mM), SAFC Biosciences, Catalog No. 59202
- Fetal Bovine Serum (FBS), SAFC Biosciences, Catalog No. 12103
- pH Meter: Orion, Model 720A

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Methods

Visual Examination and pH Determination

Four types of 10 L bioprocess bags were evaluated during this study. Eight separate bags for each type were aseptically filled with 10 L of DMEM medium (32 total bags). The bags were stored at 2 to 8 C in a darkened cold room (recommended storage conditions for liquid media). At the following time points — zero, one, four, 12, 24, 36, 48 and 60 weeks — a single bag from each type was removed from the refrigerator and visually examined for media deterioration. A sample from each bag was removed to test the pH using a standard conductivity laboratory pH meter. Indicators of deterioration included color change (the medium contains phenol red), a shift in pH, evidence of turbidity and/or formation of precipitates.

Growth Promotion Testing

At zero and 60 weeks, media samples from each bag type underwent a growth promotion study using Vero cells. To ensure that the media was not cytotoxic and retained growth promoting capabilities, cells were monitored in each media sample for three passages. The medium was supplemented with FBS and L-glutamine at the time of the test. Vero cells were seeded in T-25 flasks at $1-2 \times 10^4$ cells/cm² in each media sample (triplicate flasks for each sample). The flasks were incubated in a 5% CO₂ incubator at 36 ± 1 C for three days. The cells were harvested using standard trypsinization procedures, pooled and reseeded in each respective medium. The cultures were continued for three passages. The control medium was DMEM stored in PETG bottles which has a known stability of > 60 weeks under proper storage conditions.

Results

Over the course of 60 weeks, no visible indications of deterioration were noted in any of the bags of media. The determinations of pH also indicated little variability as shown in Figure 1. The quality control specification for pH for DMEM medium is 7.0 - 7.4, and with the exception of one, all time points were within this specification. The week 60 time point for the Stedim Flexboy bag was slightly above specification with an average pH of 7.41.

Growth promotion testing at weeks zero and 60 provide evidence that the media stored in the EVA and PE bags remained stable and retained its growth promoting capability. As seen in Figure 2, the viable cell counts per flask are similar for each medium over the course of the three passages. This trend is repeated at week 60, as seen in Figure 3, indicating that the choice of bag did not affect the growth promoting capabilities of the medium. Culture viabilities in all media exceeded 95% at both the zero and 60 week tests (Tables 1 and 2), indicating that the media was not cytotoxic.

Conclusions

Degradation or contamination of the liquid media can occur in a number of ways, including leaching of chemicals from the contact surface of the storage container and intrusion of gases or other contaminants. The visual, pH and growth promoting tests indicated no deterioration of the liquid media in any of the storage bags tested. This study indicates that DMEM medium stored in bags with either an EVA or PE fluid contact surface remained stable over a period of 60 weeks at a storage temperature of 2 to 8 C.

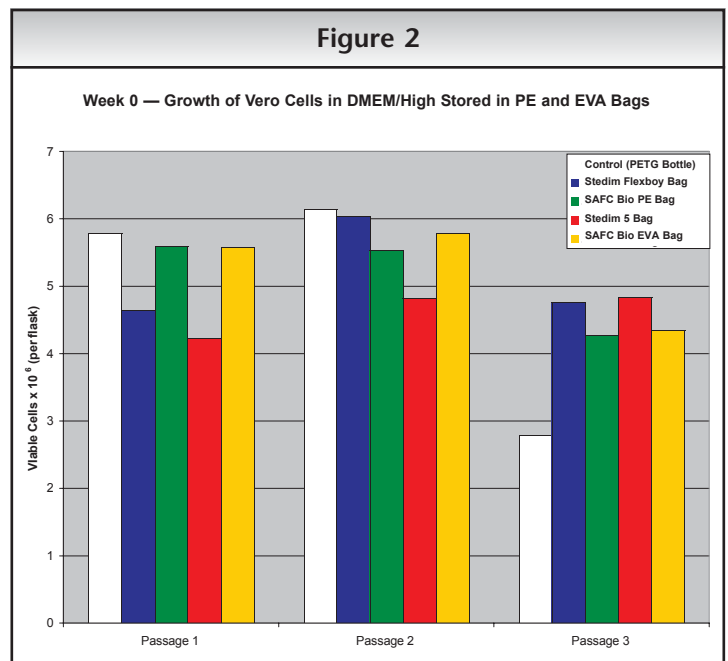
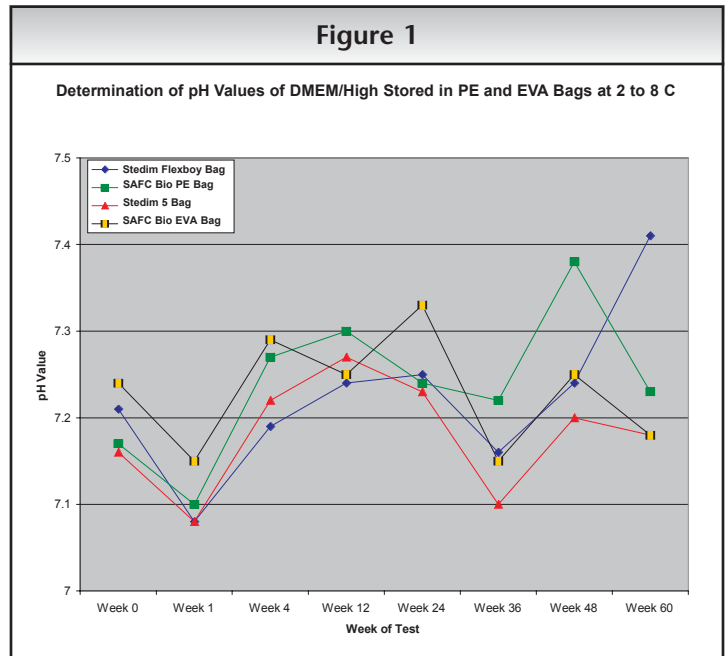


Figure 3

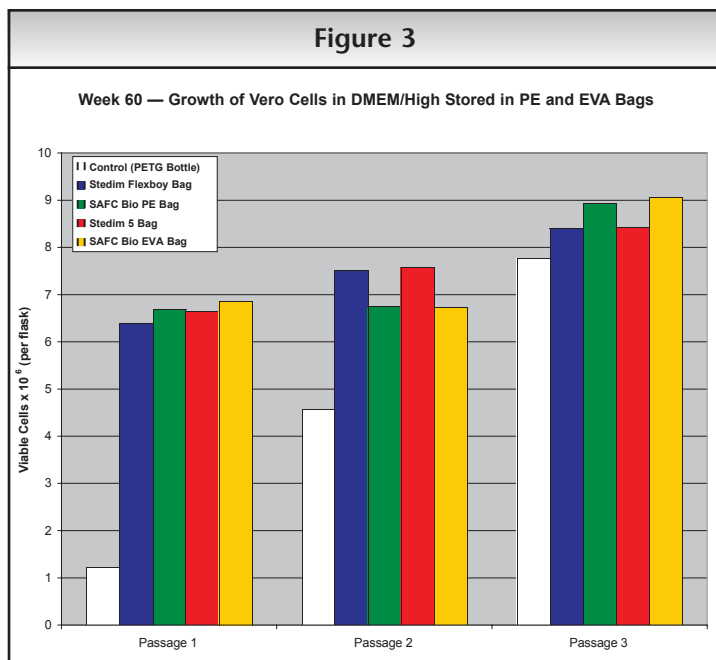


Table 1. Average Culture Viabilities During Week 0 Growth Promotion Testing

	Passage 1	Passage 2	Passage 3
Control PETG	99%	100%	100%
Stedim Flexboy Bag	100%	100%	98%
SAFC Bio PE Bag	99%	99%	95%
Stedim 5 Bag	100%	99%	98%
SAFC Bio EVA Bag	98%	100%	95%

Table 2. Average Culture Viabilities During Week 60 Growth Promotion Testing

	Passage 1	Passage 2	Passage 3
Control PETG	97%	98%	98%
Stedim Flexboy Bag	98%	98%	98%
SAFC Bio PE Bag	99%	99%	98%
Stedim 5 Bag	98%	99%	99%
SAFC Bio EVA Bag	99%	99%	99%

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