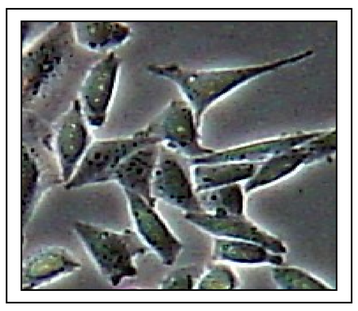

Sheff-Vax Supplement Removes the Requirement of FBS for the Culture of Vero, MDCK and BHK-21 cells

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Introduction:

The presence of Fetal Bovine Serum (FBS) in cell culture medium is necessary for the growth of some adherent and suspension mammalian cell lines. However, FBS has the inherent disadvantages of high cost, variability, ethical issues along with the risk of introducing adventitious contaminating agents. Sheff-Vax systems have been developed to help reduce or eliminate FBS or other types of animal sera from the medium of industrially relevant cell lines such as Vero, MDCK and BHK-21. The Sheff-Vax systems provide an additional advantage of simple supplementation; directly to the basal medium and an easy weaning method to help reduce or eliminate the FBS being used in the medium. The purpose of this study was to demonstrate the efficacy of the Sheff-Vax systems in reducing or eliminating the FBS in the medium of a variety of industrially relevant cell lines.

Materials and methods:

In this study, a variety of different Sheff-Vax system supplements (Sheff-Vax ACF, Sheff-Vax Plus ACF, Sheff-Vax PF ACF, Sheff-Vax Plus PF ACF, Sheff-Vax MDCK ACF and Sheff-Vax BHK PF ACF) were evaluated to determine their efficacy in reducing the FBS in the growth medium of industrially relevant cell lines.

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % FBS was used as a basal medium for the experiments performed with the two adherent cell lines; Vero (ATCC CCL-81) and MDCK (ATCC CCL-34). The cell lines were maintained in T-75 flasks incubated at 37ºC, 5 % CO_2 and routinely passaged every three to four days once the cells were found to have reached a confluency of 95 %. The experiments for Vero cells were performed in triplicate in T-25 flasks with a working volume of 5 mL and a seeding density of 0.05 x 10^6 cells/ml. For the experiments with the MDCK cells, the cells were seeded into triplicate T-75 flasks at a seeding density of 0.05 x10^6 cells/mL to 0.06 x 10^6 cells/mL with a working volume of 15 ml. Routine protocols were used for detachment of the cells from the T-flasks and seeding of the cells into new flasks to begin an experiment. A commercially available recombinant trypsin was used to detach the cells from the surface of the flasks. For Vero cells, once the serum levels were below 1.5 %, a commercially available animal component free trypsin inhibitor was used to reduce FBS carryover. Growth and viability was measured on the Nova Bioprofile Flex automated cell counter. The flasks were then incubated 37ºC, 5 % CO_2 for 3-4 days in the incubator and images of the cell morphology were obtained using the camera on the microscope, the routine procedure was followed for detachment and counting of the cells.

It was necessary to apply a range of concentrations of all the Sheff-Vax supplements initially to each of the adherent cell lines to determine the optimum concentration. All the Sheff-Vax supplements being tested were made at 100 g/L stock concentration in the basal medium being used for each of the respective cell line. Sheff-Vax supplements were added to the cell lines at a final concentration of 2, 4, 6, 8 and 12 g/L. The cells were then weaned off FBS by slowly decreasing it's concentration at each passage by 25 %. The optimum Sheff-Vax supplement concentration for each cell line was then selected based on the cell growth and viability. For Vero cells, Sheffield™ rInsulin ACF was included in the medium once the FBS levels dropped below 1.5 % to maintain high growth rate and viability. For the MDCK cells, the four conventionally available Sheff-Vax supplements were initially used to reduce the FBS in the growth medium to 0.1 %, any further decrease in the FBS level was detrimental to cell growth and viability. To address this issue and to eliminate the FBS from the growth medium, a variety of different growth factors and trace elements were analyzed in the presence of the Sheff-Vax supplements. This led to the development of a specialized version of the supplement, Sheff-Vax MDCK ACF containing growth factors, which enabled the successful elimination of the serum from the growth medium of MDCK cells.

The BHK-21 cells (ATCC CCL-10) which were internally adapted to suspension were weaned off FBS by using much higher concentrations of Sheff-Vax supplements at 8, 10 and 12 g/L. For the BHK-21 cells, using the four conventionally available Sheff-Vax supplements, it was possible to drop the FBS level to 0.5 %, any further decrease in FBS proved detrimental to the cell growth and viability. To overcome this issue and to completely eliminate the serum from the growth medium, a variety of different growth factors and trace elements were analyzed in the presence of the Sheff-Vax supplements. As a result, a specialized version, Sheff-Vax BHK PF ACF containing trace elements was developed for BHK cells. This special version was successfully used to grow the BHK-21 cells in the absence of serum in the growth medium. The BHK-21 cells were weaned off FBS by gradually decreasing the concentration by 25 %. The optimum Sheff-Vax supplement concentration was then selected based on the cell growth and viability. Glasgow Minimal Essential Medium (GMEM) was used as the basal medium for the experiments performed with BHK-21 suspension cells. Pluronic F-68 was used at 0.25 % in the medium to maintain the cells in suspension. This study was performed in triplicate 125 mL Erlenmeyer flasks with a working volume of 35 mL each and a seeding density of 0.5 x 10^6 cells/mL . The flasks with the BHK-21 cells were incubated at 37ºC, 5 % CO_2 and constant mixing at 135 rpm. The cells were passaged routinely by centrifuging appropriate volume of the cell suspension at 200 g for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in the fresh medium in the corresponding flasks to prevent any carryover of the spent medium.

Summary:

Using all four of the Sheff-Vax systems, it was possible to adapt and sustain Vero cells at 0 % FBS for multiple passages. The optimum Sheff-Vax ACF concentration was found to be 2 g/L. The cells did not show any morphological changes as compared to the control (Figure 1). The cells at 0 % FBS were reaching cell densities about half that of the 10 % FBS control and similar cell viability in the same amount of time (Figure 2). It was observed that the cells growing at 0 % FBS reached peaking densities similar to the control when allowed to grow for a longer time as compared to the control. The growth of the Vero cells at 0 % FBS was confirmed by passaging the cells in the serum-free medium for a minimum of eight passages.

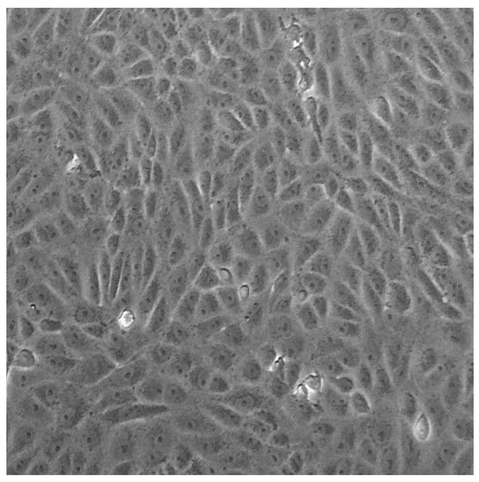
Sheff-Vax MDCK ACF which was specifically developed for use with MDCK cells and supplemented at a final concentration of 2 g/L proved to be the best supplement for FBS elimination in the MDCK cell line. When observed under the microscope the cells did not show any morphological changes as compared to the control (Figure 3). The cell growth in the absence FBS, was similar to the control (Figure 4). The growth of the MDCK cells at 0 % FBS was confirmed by passaging the cells in the serum-free medium for a minimum of eight passages.

For the BHK-21 cells, the Sheff-Vax BHK PF ACF was able to sustain the growth of the cells in the absence of FBS and reached peak cell densities similar to or better than the 10 % FBS control (Figure 5). The growth of the BHK-21 cells at 0 % FBS was confirmed by passaging the cells in the serum-free medium for a minimum of eight passages.

The study shows the high efficiency of the Sheff-Vax systems in reducing the FBS in the growth medium in these three industrially relevant cell lines. The advantage of using the Sheff-Vax systems is evident from the simple weaning method involved in adapting the cells to grow in the absence of FBS in the growth medium. The next step will be the performance of virus titer studies to ensure removing FBS will not adversely effect viral productivity in the newly adapted FBS free cell lines.

Morphology of Vero cells at 10 % FBS and 0 % FBS supplemented with Sheff-Vax ACF

(A)



(B)

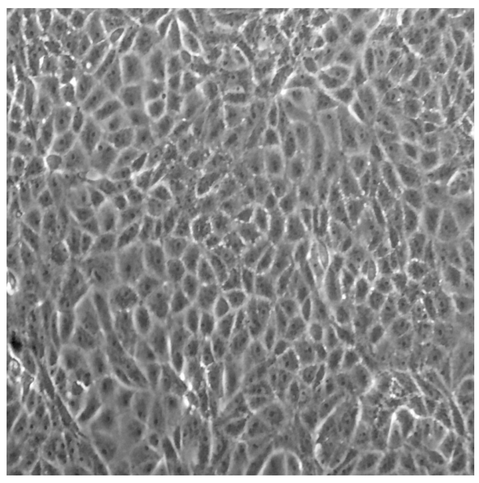


Figure 1

Images comparing the morphology of Vero cells cultured in DMEM with **(A)** 10 % FBS control and **(B)** 0 % FBS supplemented with 2 g/L Sheff-Vax ACF + 10 mg/L Sheffield™ rInsulin ACF. There was no observable morphological difference between the control cells at 10 % FBS and the cells growing at 0 % FBS. All the images were taken using a Leica inverted light microscope at 100x.

Growth profile for Vero cells in 10 % FBS compared to cells grown in Sheff-Vax ACF with DMEM at 0 % FBS

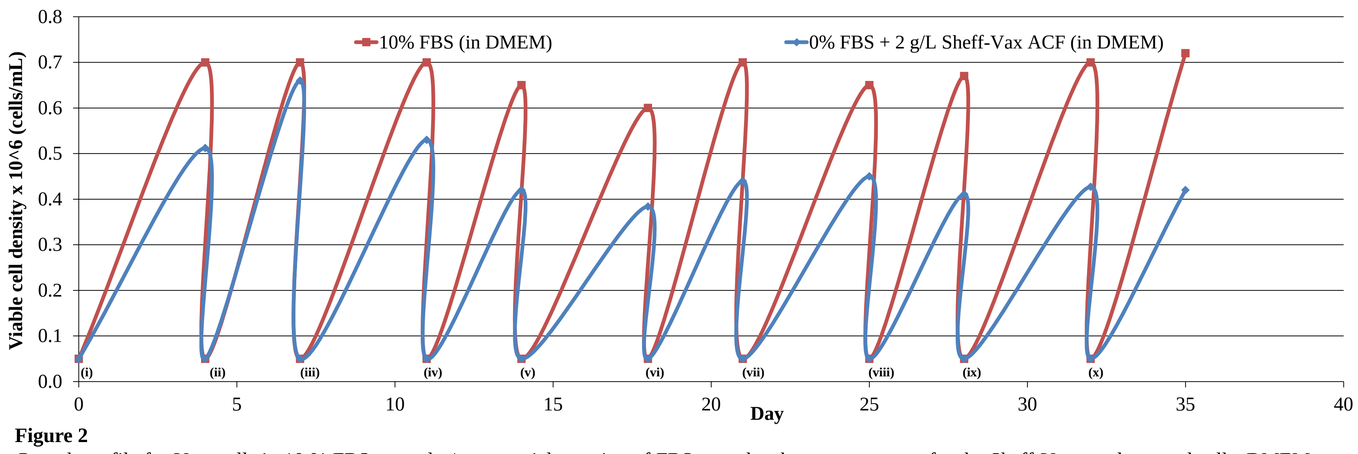
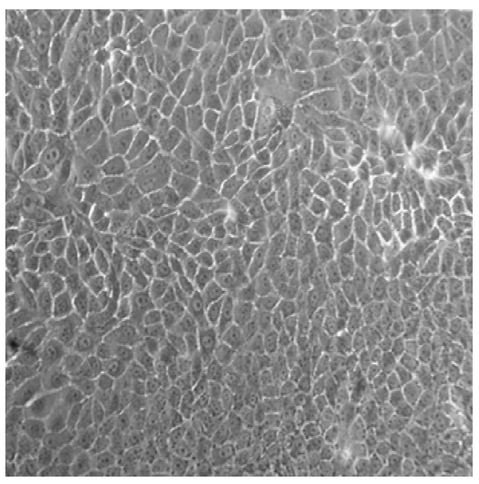


Figure 2

Growth profile for Vero cells in 10 % FBS control v/s sequential weaning of FBS at each subsequent passage for the Sheff-Vax supplemented cells. DMEM was used as the basal medium. (i) 10 % FBS, (ii) 7.5 % FBS, (iii) 5 % FBS,(iv) 2.5 % FBS, (v) 1.5 % FBS, (vi) 1 % FBS, (vii) 0.5 % FBS, (viii) 0.25 % FBS, (ix) 0.1 % FBS, (x) 0 % FBS. Using the sequential weaning method, it was possible to grow Vero cells at 0 % FBS with Sheff-Vax supplementation. The supplemented cells grew to half the peak densities that of control in same incubation time, however higher peak densities can be obtained by using longer incubation periods.

Morphology of MDCK cells at 10 % FBS and 0 % FBS supplemented with Sheff -Vax MDCK ACF

(A)



(B)

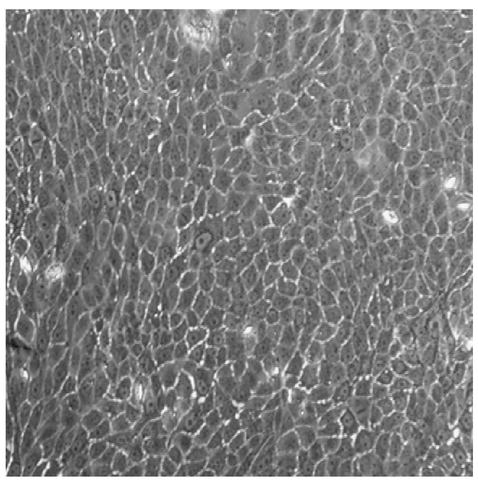


Figure 3

Images comparing morphology of MDCK cells cultured in DMEM with **(A)** 10 % FBS and **(B)** 0 % FBS supplemented with 2 g/L of Sheff-Vax MDCK ACF respectively. It was showed that no distinguished morphological change was observed when Sheff-Vax Plus ACF was used.

Growth profile for MDCK cells in 10 % FBS compared to cells grown in Sheff-Vax MDCK ACF with DMEM at 0 % FBS

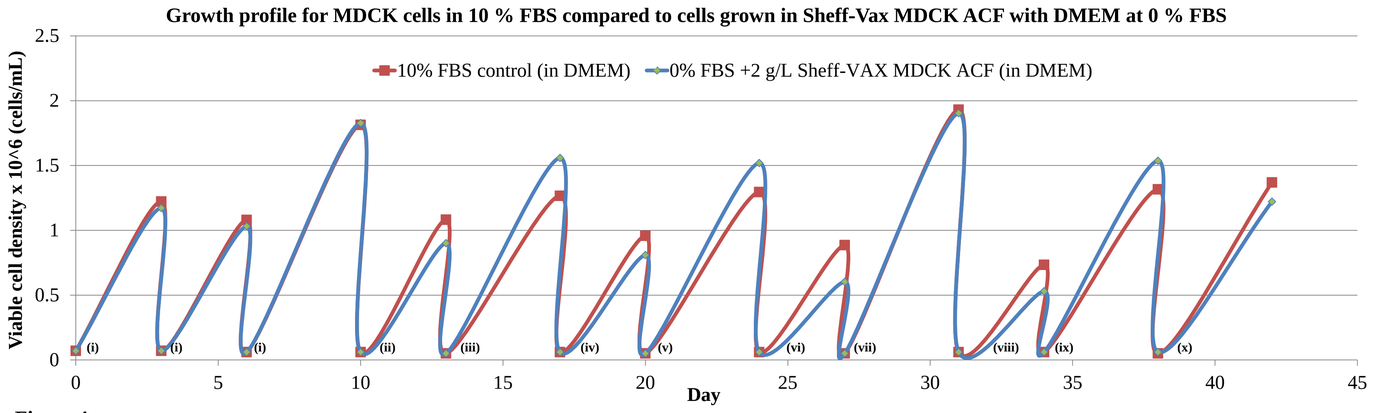


Figure 4

Growth profile for MDCK cells in 10 % FBS control v/s sequential weaning of FBS at each subsequent passage for the Sheff-Vax MDCK supplemented cells. DMEM was used as the basal medium. (i) 10 % FBS, (ii) 7.5 % FBS, (iii) 5 % FBS, (iv) 2.5 % FBS, (v) 1.5 % FBS, (vi) 0.75 % FBS, (vii) 0.5 % FBS, (viii) 0.25 % FBS, (ix) 0.1 % FBS, (x) 0 % FBS. Using the sequential weaning method, it was possible to grow the MDCK cells at 0 % FBS with Sheff-Vax MDCK supplementation. The supplemented cells grew to similar peak cell densities as that of 10 % FBS control.

BHK-21 cell growth profile in reduced FBS medium with 12 g/L Sheff-Vax BHK PF ACF with GMEM at 0 % FBS

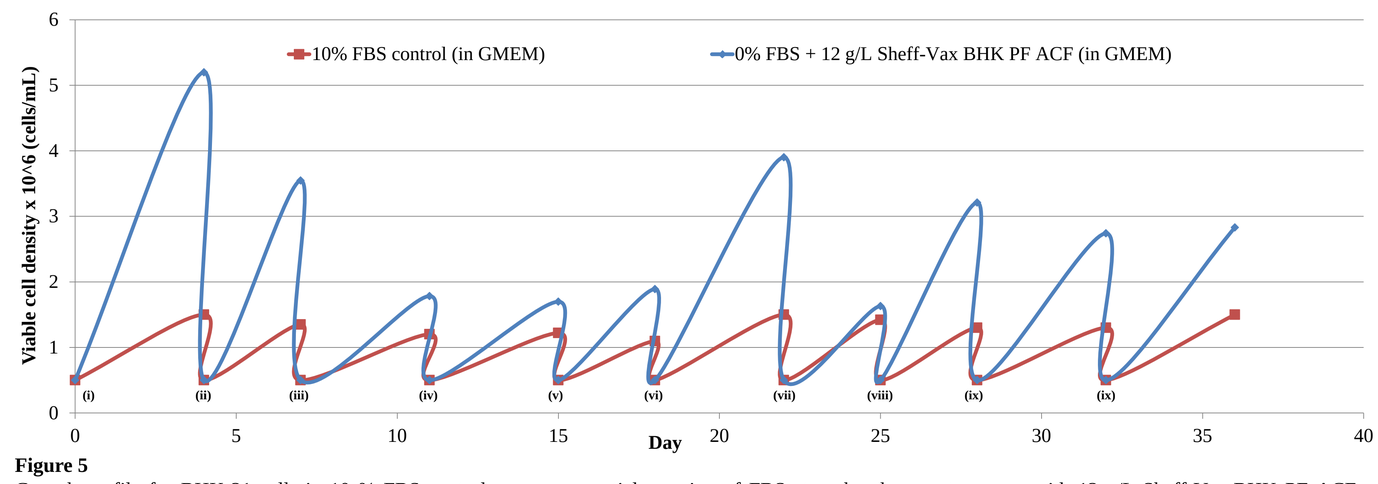


Figure 5

Growth profile for BHK-21 cells in 10 % FBS control versus sequential weaning of FBS at each subsequent passage with 12 g/L Sheff-Vax BHK PF ACF and 0.25 % Pluronic-F68. GMEM was used as the basal medium. (i) 5 % FBS, (ii) 2.5 % FBS, (iii) 1.5 % FBS, (iv) 0.75 % FBS, (v) 0.5 % FBS, (vi) 0.25 % FBS, (vii) 0.1 % FBS, (viii) 0.1 % FBS, (ix) 0 % FBS The sequential weaning method for the reduction of FBS was successful for the BHK-21 cells with the cells in reduced FBS medium with Sheff-Vax BHK ACF reaching similar peak densities as the control medium in the same time period.