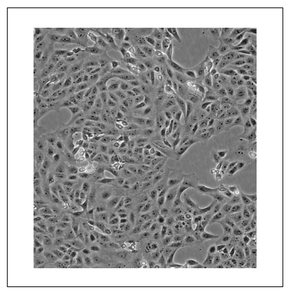

Evaluation of the use of Sheff-Vax products for Vero cells growth and rabies virus replication

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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 are animal component free (ACF) supplements that 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 adding 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 evaluate the use of Sheff-Vax products for serum reduction of Vero cells and to also analyze the effect of the Sheff-Vax products on Vero rabies virus replication.

Materials and methods:

The evaluation of the use of Sheff-Vax products for Vero cells growth and rabies virus replication was divided up into three steps;

1. Evaluation of the effect of Sheff-Vax products on rabies virus titer in cells grown at a regular FBS concentration.
2. Adaption of the Vero cells to serum free conditions using the Sheff-Vax supplements
3. Assessment of the ability of Vero cells to produce rabies virus in serum free conditions supplemented with Sheff-Vax.

1. Studying the effect of Sheff-Vax products on rabies virus titer

The effect of four products, Sheff-Vax ACF, Sheff-Vax Plus ACF, Sheff-Vax PF ACF and Sheff-Vax Plus PF ACF VP on virus production was investigated in T-25 flask cultures. Vero cells were grown in MEM + 10 % FBS at a 10 ml working volume with a seeding density of 2 x 10^6 cells per T-25 flask. The experiments were performed in triplicate. The cells were infected at the start of the cultures at an MOI of 0.1 with the LP2061 rabies virus strain. Four days post infection, the medium was exchanged with M199 medium supplemented with various concentrations of each Sheff-Vax supplement to be evaluated and the flasks were incubated at 34 °C. Each Sheff-Vax supplement was tested at 2, 4 and 6 g/L, while the Sheff-Vax PF ACF VP was also tested at 8 g/l. Infection under standard conditions (M199 + 0.2 % BSA) was considered as a positive control. Three harvests were conducted at 3 day intervals for each condition tested and titers were analyzed (Fig 1).

2. Adaption of Vero cells to serum free conditions using Sheff-Vax products

The use of Sheff-Vax Plus PF ACF to reduce serum levels in Vero cultures was next investigated. The experiments were performed in T-25 flasks with a working volume of 10 ml and a seeding density of 2 x 10^6 cells per T-25 flask. The starting media consisted of DMEM supplemented with 10 % FBS. This media was supplemented with 2 g/l Sheff-Vax Plus PF ACF & 10 mg/l Sheffield rInsulin ACF. The cells were then adapted by the gradual reduction in FBS from 10 % to 0 % over 17 passages (Fig 2(a)). The adapted cells were cryopreserved in liquid nitrogen in the medium DMEM + 10 % DMSO + 0.1 % methylcellulose. Cells were kept in liquid nitrogen for several weeks, then one ampoule was defrosted and the cells were amplified (Fig 2(b)).

3. Assessing the ability of Vero cells to produce rabies virus in serum free conditions with Sheff-Vax products

The Vero cells that were adapted to serum free conditions were then evaluated to ascertain if these cells could still produce economically viable viral yield and whether the Sheff-Vax supplements affected rabies titer. Defrosted Vero cells were grown in DMEM + 2 g/l Sheff-Vax Plus PF ACF + 10 mg/l Sheffield rInsulin ACF in T-25 flasks. Vero cells were infected with LP2061 rabies strain at day 0 and at an MOI of 0.1. Four days post infection, cells were washed with DMEM and the medium was exchanged with the medium to be tested; flasks were incubated at 34 °C. Three harvests were conducted at 3 day interval for each condition tested. Experiments were conducted in T-flasks in duplicate. DMEM supplemented Sheff-Vax ACF at 2 g/l, Sheff-Vax Plus ACF at 2 g/l, Sheff-Vax Plus PF ACF VP at 7.6 g/l and Sheff-Vax PF ACF at 8 g/l were all tested based on a previous DOE (data not shown) studying the effect of individual Sheff-Vax products on rabies virus replication in Vero cells. A mixture of these products were also tested based on the DOE results. Infection in MEM + 0.2 % BSA and in DMEM + 2 g/l Sheff-Vax Plus PF ACF + 10 mg/l Sheffield rInsulin ACF (Kerry's media) were considered the positive controls (Fig 3).

Summary:

The first step in this study was to analyze the effect of Sheff-Vax products on rabies virus titers in Vero cells. The cells were initially grown up in MEM + 10 % FBS, and the media was exchanged 4 days post infection with M199 media either supplemented with Sheff Vax products at 2, 4 & 6 g/l (Sheff-Vax Plus PF ACF VP was also tested at 8 g/l) or supplemented with 0.2 % BSA as a control. Figure 1 shows the total virus production for each condition and it is clear that the Sheff-Vax products result in greater rabies virus titers compared to the 0.2 % BSA control. All of the Sheff-Vax products, at each concentration tested, resulted in greater titer yields compared to the 0.2 % BSA control

The next step was to ascertain the effect of FBS reduction on the growth of the Vero cells. DMEM (base medium) supplemented with Sheff-Vax Plus PF ACF at 2 g/l + 10 mg/l Sheffield rInsulin ACF allowed the Vero cells to be successfully weaned from 10 % FBS to 0 % FBS (Figure 2(a)). The cells were then frozen and kept in LN2 for several weeks. After introducing the cells back into culture, growth reached a level comparable to the 10 % FBS control after a number of passages (Figure 2(b)). This is an example of how supplementation with 2 g/l Sheff-Vax Plus PF ACF + 10 mg/l Sheffield rInsulin ACF can completely eliminate FBS from Vero medium while still maintaining adequate growth.

Lastly, the effect of a number of Sheff-Vax products on titer in the cells adapted to 0 % FBS was analyzed. The cells were initially grown in DMEM + 2 g/l Sheff-Vax Plus PF ACF + 10 mg/l Sheffield rInsulin ACF. Four days post infection, cells were washed with DMEM and the medium was exchanged with the medium to be tested (the Sheff-Vax conditions chosen to be tested were based on a previous DOE experiment-not shown). The use of DMEM supplemented with Sheff-Vax ACF and Sheff-Vax Plus ACF at 2 g/l resulted in the highest virus titers; Sheff-Vax Plus PF ACF VP and Sheff-Vax PF ACF resulted in slightly lower production of rabies virus although still higher than the positive control (MEM + 0.2 % BSA). The use of the same medium for cell replication and virus production (Sheff-Vax Plus PF ACF at 2 g/l + 10 mg/l Sheffield rInsulin ACF) resulted in a higher virus titer when compared to the positive control medium and to the mixture of Sheff-Vax products. Overall, all of the Sheff-Vax supplements tested resulted in a higher total rabies virus titer compared to the positive control.

Conclusion:

This study demonstrated the benefits of using the Sheff-Vax supplement range in a number of aspects of virus production. Not only did the use of Sheff-Vax supplements increase rabies virus titer when used in a regular FBS supplemented growth media, the Sheff-Vax supplements can also be used to completely eliminate the use of FBS in the media for growth of Vero cells. In addition, the serum free Vero cells can then be used in combination with a range of Sheff-Vax products to increase rabies virus titer when compared to a 0.2 % BSA control. In conclusion, the Sheff-Vax supplements are a valuable tool that can be used to both eliminate FBS from viral production medium, while also enhancing viral production in Vero cells.

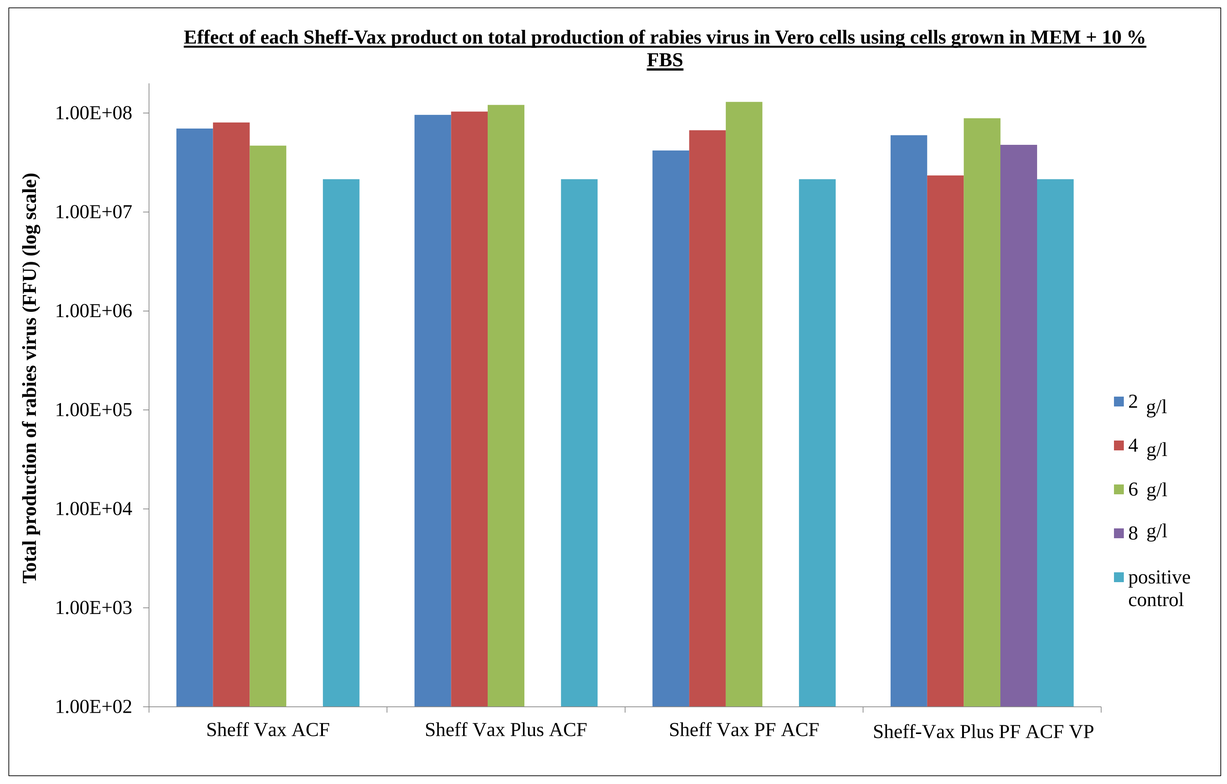

Figure 1: Effect of Sheff-Vax products on rabies virus titer. The use of a number of Sheff-Vax products and their effect on titers were analyzed using Vero cells grown in MEM + 10 % FBS. Four days after infection, the medium was exchanged for the medium to be tested. Titers were compared to an M199 + 0.2 % BSA positive control. The Sheff-Vax products were tested at 2, 4 , 6 and 8 g/L. At 8 g/L the supplements were toxic to the Vero cells with the exception of Sheff-Vax PF ACF VP. It is clear that all of the Sheff-Vax products, at each concentration tested, resulted in a greater virus titer yield when compared to the 0.2 % BSA control.

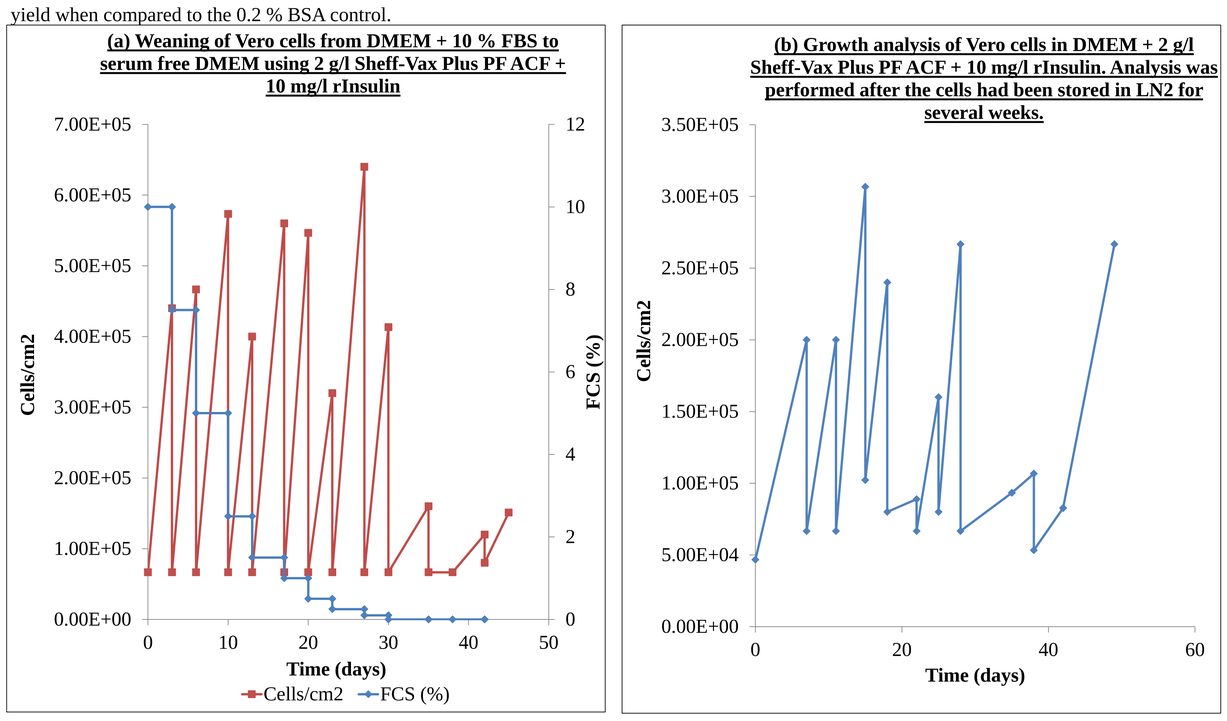

Figure 2: Adaption of Vero cells to 0 % FBS. The starting media consisted of DMEM with 10 % FBS. This media was then supplemented with 2 g/l Sheff-Vax Plus PF ACF + 10 mg/L Sheffield rInsulin ACF. The Vero cells were then gradually weaned over a number of passages to completely eliminate FBS from the medium (Figure2 (a)). The cells were then cryopreserved in DMEM + 10 % DMSO + 0.1 % methylcellulose. After a number of weeks in liquid nitrogen the cells were put back into culture and after a few passages, the growth density was comparable to the 10 % FBS control (Figure 2 (b)).

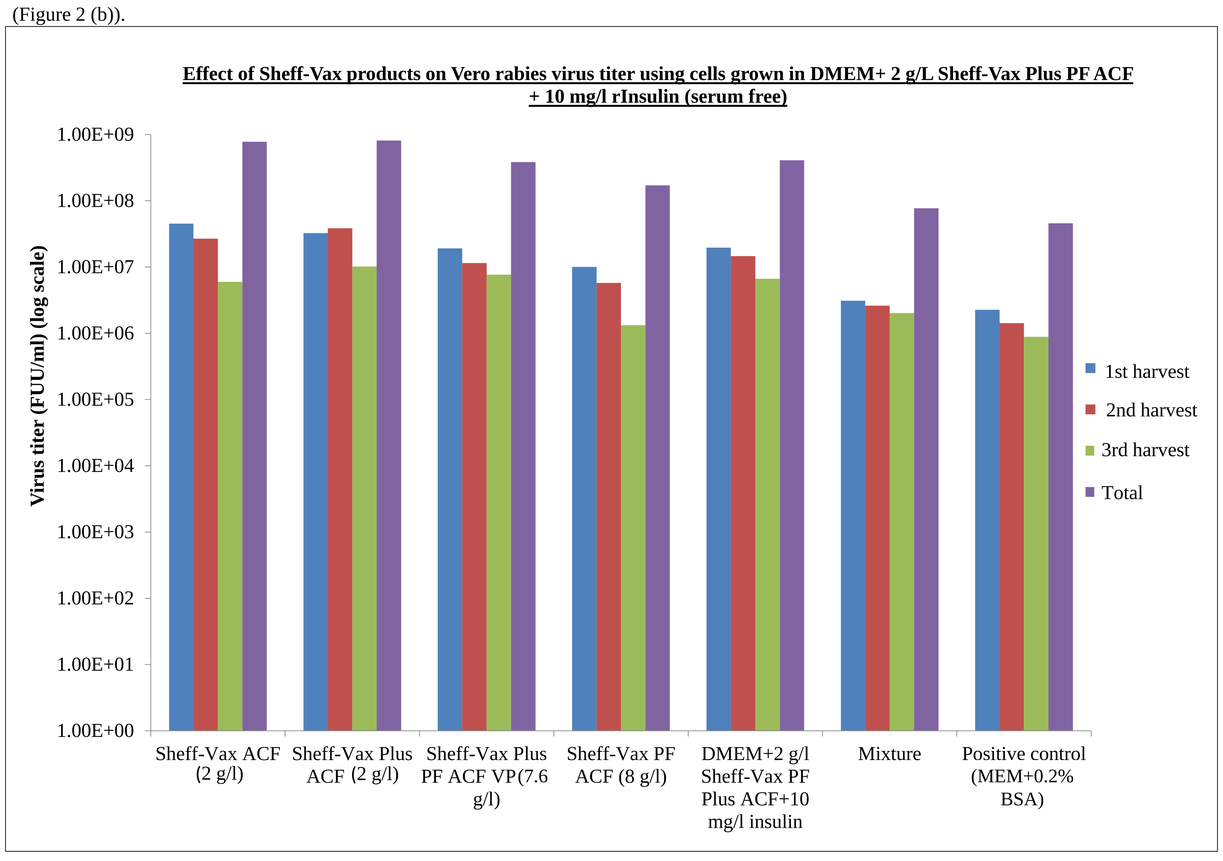

Figure 3 Effect of FBS elimination & the use of Sheff-Vax products on rabies virus titer. The use of a number of Sheff-Vax products and their effect on titers were analyzed using the cells adapted to serum free medium using 2 g/l Sheff-Vax Plus PF ACF + 10 mg/l Sheffield rInsulin ACF. Titers were compared to an MEM + 0.2 % BSA positive control. All Sheff-Vax products tested resulted in a higher rabies virus titer than the positive control. Removing FBS had no effect on the ability of the Vero cells to produce virus and actually increased virus titer.