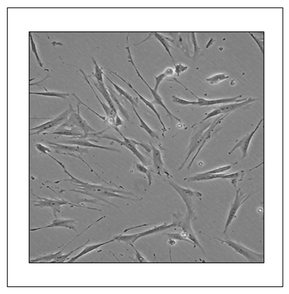

Evaluation of the use of Sheff-Vax products for MRC-5 cells growth and measles virus replication

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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 MRC-5 cells and to also analyze the effect of the Sheff-Vax products on MRC-5 measles virus replication.

Materials and methods:

The evaluation of the use of Sheff-Vax products for MRC-5 cells growth and measles virus replication was divided up into three steps;

1. Evaluation of the effect of Sheff-Vax products on measles virus titer in cells grown at a regular FBS concentration.
2. Adaption of the MRC-5 cells to low FBS conditions using the Sheff-Vax supplements
3. Assessment of the ability of MRC-5 cells to produce measles virus in low FBS conditions supplemented with Sheff-Vax.

1. Studying the effect of Sheff-Vax products on measles 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. MRC-5 cells (ATCC CCL-171) were grown in MEM + 5 % 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 0.001 with the AIK-C measles virus strain. Three 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. Infection under standard conditions (M199 + 0.5 % 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).

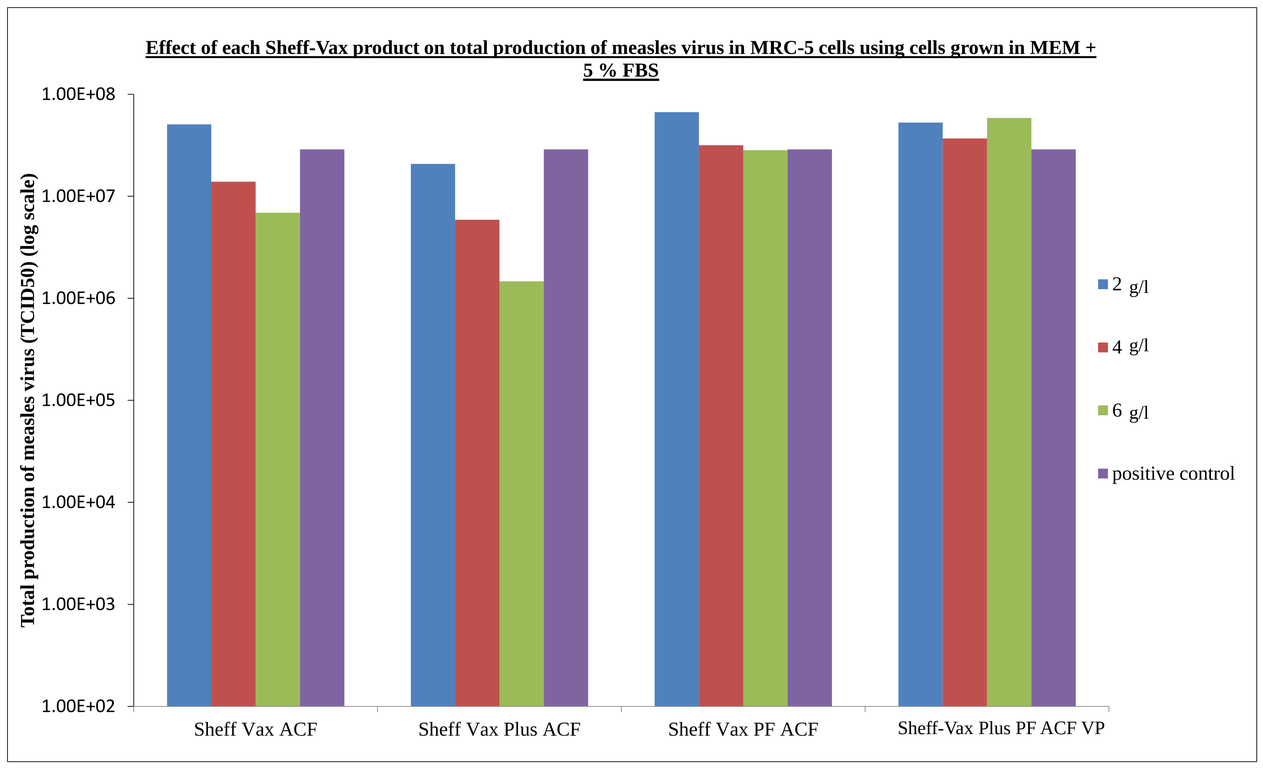

Figure 1: Effect of Sheff-Vax products on measles virus titer. The use of a number of Sheff-Vax products and their effect on titers were analyzed using MRC-5 cells grown in MEM + 5 % FBS. Three days after infection, the medium was exchanged for the medium to be tested. Titers were compared to an M199 + 0.5 % BSA positive control. The Sheff-Vax products were tested at 2, 4 & 6 g/l,. It is clear that the majority of Sheff-Vax products, at each concentration tested resulted in a comparable or greater virus titer yield when compared to the 0.5 % BSA control.

2. Adaption of MRC-5 cells to low FBS conditions using Sheff-Vax products

The use of Sheff-Vax Plus ACF to reduce serum levels in MRC-5 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 5 % FBS. This media was supplemented with 2.5 g/l Sheff-Vax Plus ACF & 10 mg/l Sheffield rInsulin ACF. The cells were then adapted by the gradual reduction in FBS from 5 % to 1.5 % over 9 passages (Fig 2(a)). The adapted cells were cryopreserved in liquid nitrogen in the medium DMEM + 1.5 % FBS + 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF + 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 MRC-5 cells to produce measles virus in low FBS conditions with Sheff-Vax products

The MRC-5 cells that were adapted to low (1.5 % FBS) conditions were then evaluated to ascertain if these cells could still produce economically viable viral yield and whether the Sheff-Vax supplements affected measles titer. Defrosted MRC-5 cells were grown in DMEM + 1.5 % FBS + 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF in T-25 flasks. MRC-5 cells were infected with AIK-C measles strain at day 4 and at an MOI of 0.001. 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 6 g/l, Sheff-Vax Plus ACF at 2 g/l, Sheff-Vax Plus PF ACF VP at 4 g/l and Sheff-Vax PF ACF at 2 g/l were all tested based on a previous DOE (data not shown) studying the effect of individual Sheff-Vax products on measles virus replication in MRC-5 cells. A mixture of these products were also tested based on the DOE results. Infection in MEM + 0.5 % BSA and in DMEM + 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF (Kerry's media) were considered the positive controls (Fig 3).

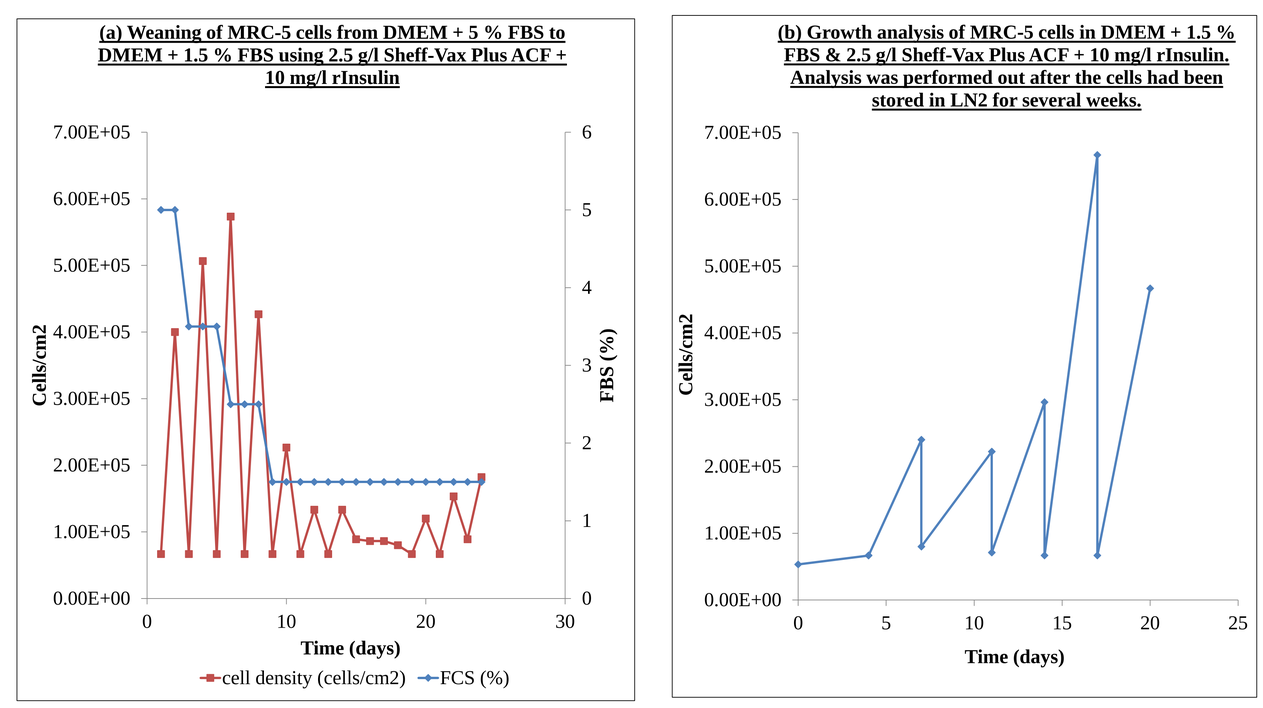

Figure 2: Adaption of MRC-5 cells to 1.5 % FBS. The starting media consisted of DMEM with 5 % FBS. This media was then supplemented with 2.5 g/l Sheff-Vax Plus ACF + 10 mg/L Sheffield rInsulin ACF. The MRC-5 cells were then gradually weaned to a reduced level of FBS (1.5 %) over a number of passages (Figure2 (a)). The cells were then cryopreserved in DMEM + 1.5 % FBS + 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF +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 5 % FBS control (Figure 2 (b)).

Summary:

The first step in this study was to analyze the effect of Sheff-Vax products on measles virus titers in MRC-5 cells. While the cells were initially grown up in MEM + 5 % FBS, the media was exchanged 3 days post infection with M199 media either supplemented with Sheff Vax products at 2, 4 & 6 g/l or supplemented with 0.5 % BSA as a control. Figure 1 shows the total virus production for each condition and it is clear that the virus titers in the Sheff-Vax supplemented media were at least comparable to, if not greater than the 0.5 % BSA control. The Sheff-Vax PF ACF & Sheff-Vax Plus PF ACF VP showed the highest overall production levels of the Sheff-Vax products and were also higher overall at each concentration tested when compared to the 0.5 % BSA control. The optimum concentration of the Sheff-Vax ACF & Sheff-Vax Plus ACF tested was 2 g/l, which resulted in comparable titers to the 0.5 % BSA control.

The next step was to ascertain the effect of FBS reduction on the growth of the MRC-5 cells. DMEM (base medium) supplemented with Sheff-Vax Plus ACF at 2.5 g/l + 10 mg/l Sheffield rInsulin ACF allowed the MRC-5 cells to be successfully weaned from 5 % FBS to 1.5 % 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 5 % FBS control after a number of passages (Figure 2(b)). This is an example of how supplementation with 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF can drastically reduce the FBS requirement of MRC-5 cells while still maintaining adequate growth.

Lastly, the effect of a number of Sheff-Vax products on titer in the cells adapted to 1.5 % FBS was analyzed. The cells were initially grown in DMEM + 1.5 % FBS + 2.5 g/l Sheff-Vax Plus 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). Among the Sheff-Vax products tested, DMEM enrichment with either Sheff-Vax Plus PF ACF VP at 4 g/l, She-Vax PF ACF at 2 g/L and Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF (Kerry's medium) enhanced the virus titer compared to the 0.5 % BSA control.

Conclusion:

This study successfully 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 measles virus titer when used in a regular FBS supplemented growth media, the Sheff-Vax systems could also significantly reduce the requirement for FBS to cultivate MRC-5 media without negatively affecting growth. In addition, the lower FBS MRC-5 cells can then be used in combination with a range of Sheff-Vax products to not only match, but also increase measles virus titer when compared to a 0.5 % BSA control. In conclusion, the Sheff-Vax supplements are a valuable tool that can be used to both reduce FBS in the medium, while also maintaining or in some cases enhancing viral production in MRC-5 cells.

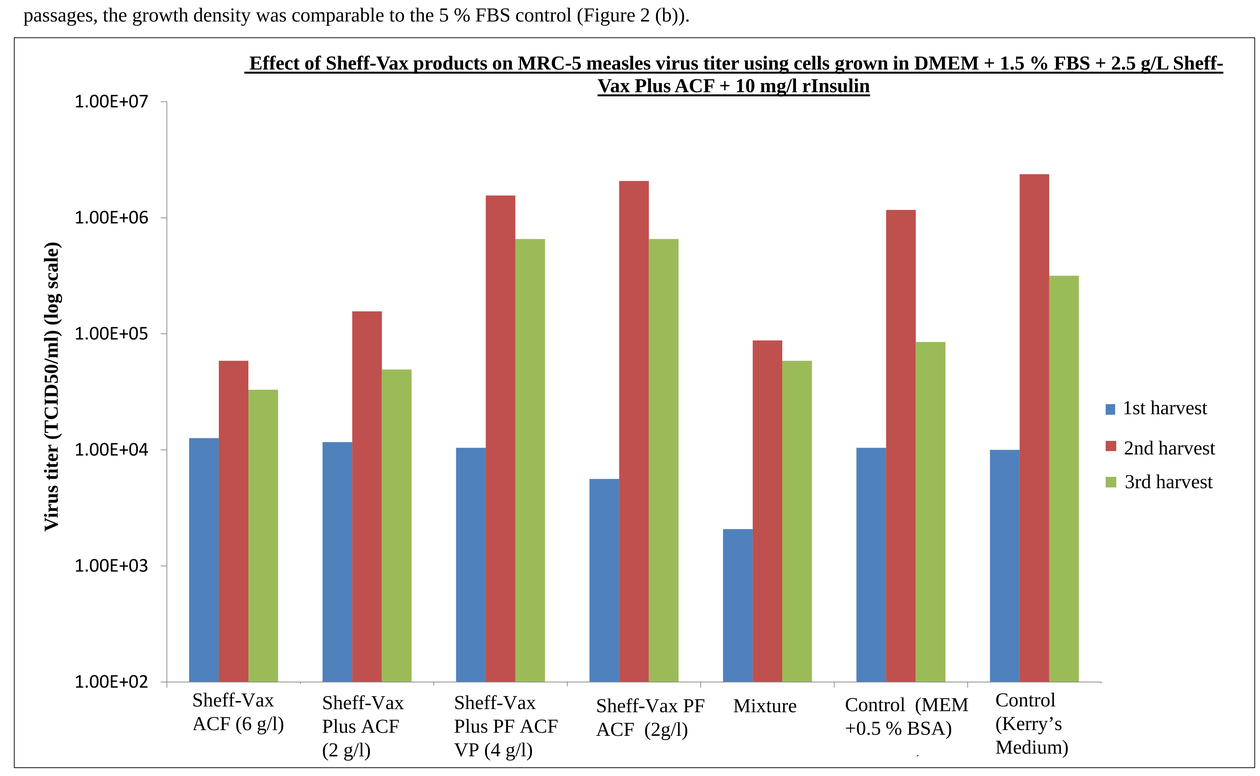

Figure 3 Effect of FBS elimination & the use of Sheff-Vax products on measles virus titer. The use of a number of Sheff-Vax products and their effect on titers were analyzed using the cells adapted to 1.5 % FBS using 2.5 g/l Sheff-Vax Plus ACF + 10 mg/l Sheffield rInsulin ACF. Titers were compared to an MEM + 0.5 % BSA positive control.