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The use of specialised Sheff-Vax ACF supplements for BHK-21/SUSP/ARRIAH cell cultivation and FMDV reproduction

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SUMMARY

Compliance with the existing purity and safety requirements for immunobiologicals can be effectively achieved by the use of serum-free nutrient media and specialised supplements of non-animal origin. The paper shows the possibility of using Sheff-Vax ACF® supplements (Kerry, Inc., Ireland) for BHK-21/SUSP/ARRIAH cell cultivation and FMDV reproduction. By passage 7, cell concentration and growth rate with Sheff-Vax Plus PF ACF were found to be 40–60% higher than with Sheff-Vax PF ACF and Sheff-Vax Plus ACF. No differences were observed as regards changes in pH. During FMDV reproduction in the cells, it was found that the number of 146+75S components in the test samples containing 1 million cells was 2.3–2.4 higher compared to the controls. Cells cultured with the use of Sheff-Vax Plus PF ACF supplement had normal morphology and multiple dynamic protrusions. In the presence of this supplement, growth rate and suspension concentration in the test and control samples became equal by passage 7. The number of immunogenic components of FMDV reproduced in the cells grown using Sheff-Vax Plus PF ACF was 20–30% higher than in the cells grown using other supplements. BHK-21/SUSP/ARRIAH cell concentration and growth rate in the presence of specialised supplements were found to be lower than those in the control samples with serum and blood protein hydrolysate added to the nutrient medium. The virus yield from 1 million cells was higher in the culture grown using Sheff-Vax ACF supplements. Sheff-Vax Plus PF ACF was found to be the most suitable for BHK-21/SUSP/ARRIAH cell cultivation and FMDV reproduction in the said cells out of the three tested supplements.

Keywords: Sheff-Vax supplements, growth rate, BHK-21/SUSP/ARRIAH cells, foot-and-mouth disease virus (FMDV).

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Использование специализированных добавок Sheff-Vax ACF для культивирования клеток BHK-21/SUSP/ARRIAH и репродукции вируса ящура

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РЕЗЮМЕ

Предъявляемые в настоящее время к иммунобиологическим препаратам требования чистоты и безопасности могут быть эффективно достигнуты при использовании бессывороточных питательных сред и специализированных добавок неживотного происхождения. В данной работе показана возможность применения добавок Sheff-Vax ACF®, производимых компанией Kerry, Inc. (Ирландия), для культивирования культуры клеток ВНК-21/SUSP/ARRIAH и репродукции вируса ящура. Было отмечено, что к седьмому пассажу в присутствии добавки Sheff-Vax Plus PF ACF концентрация клеток и кратность прироста были выше на 40–60%, чем при внесении добавок Sheff-Vax PF ACF и Sheff-Vax Plus ACF. Не обнаружено различий в изменении водородного показателя. При репродукции вируса ящура в полученных клетках определили, что в опытных образцах с 1 млн клеток 146+75S компонентов было больше в 2,3–2,4 раза по сравнению с контролем. При культивировании с добавкой Sheff-Vax Plus PF ACF клетки имели нормальную морфологию, множество динамических выростов. В присутствии данной добавки к седьмому пассажу такие показатели, как кратность прироста и концентрация суспензии, в контрольных и опытных образцах выравнивались. Количество иммуногенных компонентов вируса ящура, репродуцированного в клетках с указанной добавкой, было выше на 20–30%, чем в клетках, выросших с применением других добавок. Установлено, что концентрация клеток линии ВНК-21/SUSP/ARRIAH и кратность прироста в присутствии специализированных добавок была меньше, чем в контрольных образцах с добавлением сыворотки и гидролизата белков крови в питательную среду. При этом выход вируса с 1 млн клеток был выше в культуре, выросшей при внесении добавок Sheff-Vax ACF. Из трех исследованных добавок наиболее приемлемой для культивирования линии ВНК-21/SUSP/ARRIAH и репродукции вируса ящура в полученных клетках была Sheff-Vax Plus PF ACF.

Ключевые слова: Добавки Sheff-Vax, кратность прироста, клетки ВНК-21/SUSP/ARRIAH, вирус ящура.

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INTRODUCTION

A nutrient medium helps maintain cell viability and supports their growth. It serves as a source of nutrients, growth factors and hormones, as well as regulates culture pH and osmotic pressure.

There are several types of media according to whether the growth of cells requires the presence of serum: basal media that require supplementation with 10% serum; advanced media that require supplementation with 1–5% serum; serum-free media that do not require supplementation with serum [1, 2].

The use of blood serum in cell culture has some significant drawbacks. For most tissues, this component is not the body fluid with which they had contact in the original tissue; therefore, serum promotes fibroblast growth, but inhibits the growth of epidermal keratinocytes. Besides, serum can be cytotoxic due to polyamine oxidase that has an effect on polyamines (spermine, spermidine) being the secretory products of rapidly proliferating cells (fetal serum contains relatively high levels of such enzymes). The drawbacks also include a significant serum composition variability in different batches; the amount of specific growth factors in sera can be insufficient, making it necessary to add them to cell cultures. Serum is often contaminated with viruses, many of which, though being not harmful for the cell culture, represent an additional uncontrollable factor [2].

The existing purity and safety requirements for immunobiologicals can be effectively met only by means of serum-free technology. Therefore, intensive studies have been carried out during the past two decades to develop serum-free nutrient media and specialised non-animal supplements, with their formulas being the intellectual property of companies and unavailable for common use. Such media have certain advantages such as improved reproducibility of test results due to the high stability of medium composition; decreased risk of viral, fungal, mycoplasma contamination of cell cultures; facilitation of cell metabolite removal; reduced effect of additional proteins on biological test results; the absence of cytotoxicity [3, 4].

Kerry, Inc. (Ireland) has developed several types of specialised Sheff-Vax ACF® supplements that contain milk product, egg, wheat, peanut derivatives, fish and mollusc products, etc. and differ in mineral and amino acid composition, growth factor concentration and intact protein content.

The aim of the study was to examine the possibility of using specialised serum-free Sheff-Vax ACF supplements for BHK-21/SUSP/ARRIAH cell suspension culture and FMDV reproduction.

MATERIALS AND METHODS

Cell line. A continuous suspension line of neonatal Syrian hamster kidney cells (BHK-21/SUSP/ARRIAH cell line) was used in the study [5].

Specialised supplements. The following non-animal Sheff-Vax ACF supplements (Kerry, Inc., Ireland) were used in the study: Sheff-Vax Plus PF ACF (supplement 1); Sheff-Vax PF ACF (supplement 2); Sheff-Vax Plus ACF (supplement 3).

Specialised supplements at a concentration of 10 g/dm^3 were added to the cell growth medium.

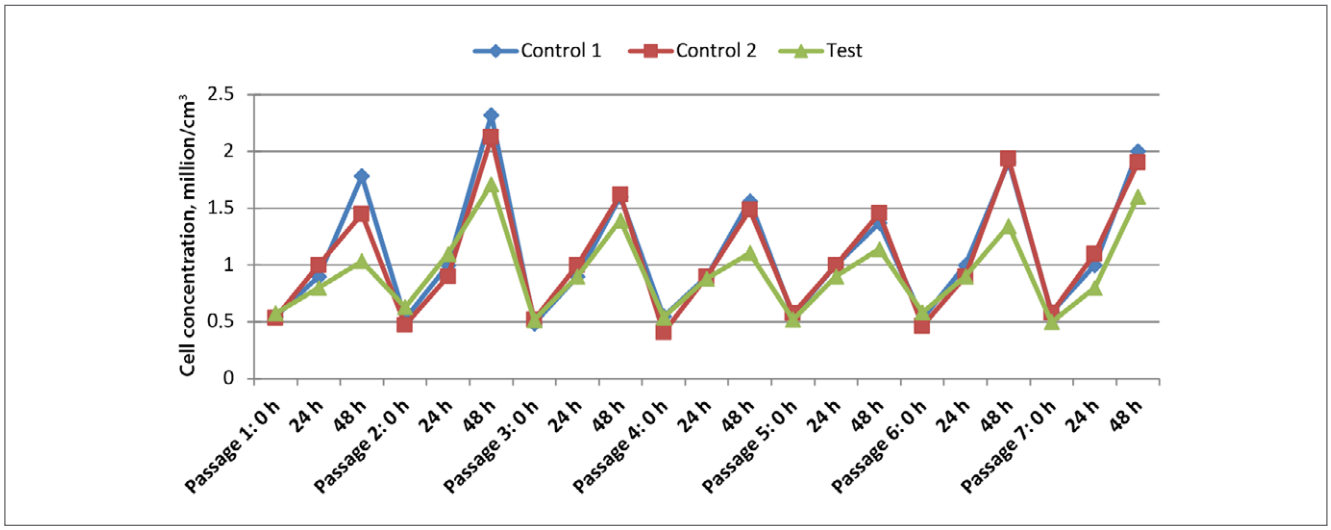

Fig. 1. BHK-21/SUSP/ARRIAH cell concentration dynamics in the presence of specialised Sheff-Vax Plus PF ACF supplement (No. 1)

Рис. 1. Динамика изменения концентрации клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax Plus PF ACF (№ 1)

The nutrient medium used to grow cells was prepared according to the Procedure for production of adsorbed polyvalent and monovalent vaccine against foot-and-mouth disease (based on the virus grown in BHK-21 cells), but no serum was added.

Bovine serum. Fetal bovine serum (Serana, Germany) at a concentration of 5% was used for the tests.

Growth rate was determined as the ratio between the final and initial cell concentrations within one passage (within 48 hours).

Cell line adaptation. At the initial stage of adaptation, cells were collected from suspension contained in the 5% serum-supplemented medium. BHK-21/SUSP/ARRIAH cell line adaptation was carried out during seven successive passages. Sodium hydrogen carbonate solution (7.5%) was used for pH adjustment 24 hours after reseeding.

The following controls were used: control 1 – a medium with constant serum content (5%), control 2 – a medium with serum percentage reduced by half with each successive passage.

A medium containing a specialised Sheff-Vax ACF supplement at a concentration of 10 g/dm^3 was used as a test medium. Serum content in the test medium was also reduced by half with each successive passage.

Viscosity. To achieve the desired viscosity, Pluronic F-68, a polymeric component, at a final concentration of 0.125% was used; it was added to control 2 and test samples from passage 2 on.

Cell infection with FMDV. Culture FMDV Asia-1/Tajikistan/2011 strain at a dose of 1.0 $TCID_{50}$/cell was used to infect suspension BHK-21/SUSP/ARRIAH cells. Passage 7 cell suspension was poured into roller bottles and, where necessary, diluted with the medium to reach a volume of 400–600 cm^3 and a cell concentration of 1.5×10^6 cells/cm^3. FMDV reproduction took place during 16 hours and was followed by the virus inactivation and suspension purification.

Cytochemical study of BHK-21/SUSP/ARRIAH cell morphology was conducted using the luminescence microscope ML-2B. Native preparations were stained with 0.001% acridine orange solution. Photographs were taken using Leica camera and Zeiss, Olympus, Leica microscopes.

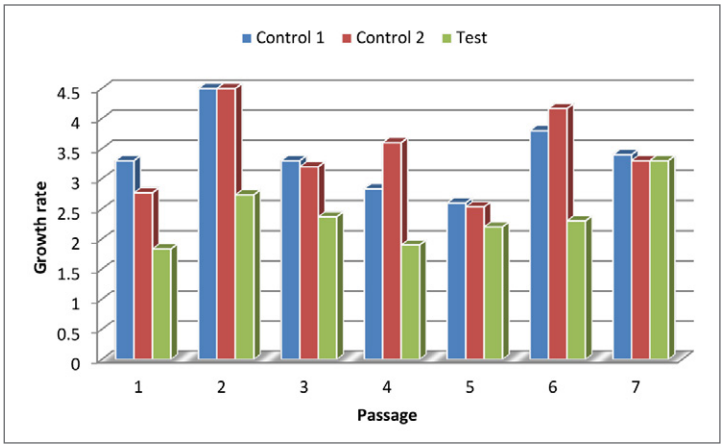

Fig. 2. BHK-21/SUSP/ARRIAH cell growth rate dynamics in the presence of specialised Sheff-Vax Plus PF ACF supplement (No. 1)

Рис. 2. Динамика изменения кратности прироста клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax Plus PF ACF (№ 1)

Viral antigen inactivation and purification. FMDV was inactivated using a 15–20% solution of aminoethylethylenimine . To remove ballast proteins, in particular FMDV non-structural proteins, from the inactivated antigen suspension, a 0.007% solution of Polysept (polyhexamethylene guanidine) was used, with subsequent decanting of supernatant.

Statistical processing of data. Numerical data were statistically processed by generally accepted methods of variation statistics using a personal computer and Microsoft Excel software.

RESULTS AND DISCUSSION

During the first stage of the study, the dynamics of BHK-21/SUSP/ARRIAH cell concentration was examined

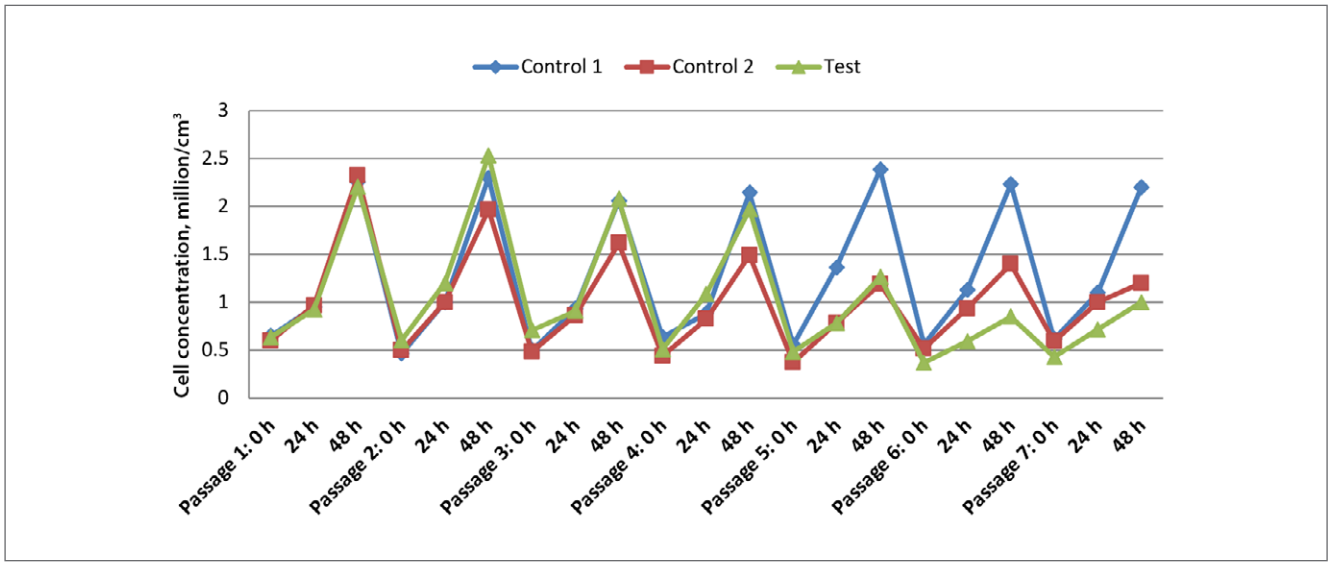

Fig. 3. BHK-21/SUSP/ARRIAH cell concentration dynamics in the presence of specialised Sheff-Vax PF ACF supplement (No. 2)

Рис. 3. Динамика изменений концентрации клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax PF ACF (№ 2)

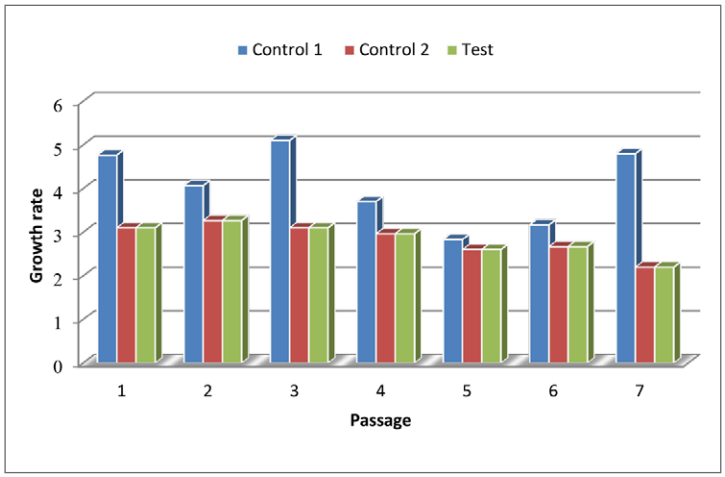

Fig. 4. BHK-21/SUSP/ARRIAH cell growth rate dynamics in the presence of specialised Sheff-Vax PF ACF supplement (No. 2)

Рис. 4. Динамика изменений кратности прироста клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax PF ACF (№ 2)

at different passages in the presence of specialised Sheff-Vax Plus PF ACF supplement (No. 1). The results are presented in Figures 1 and 2.

It was found that cell concentration in the control samples at the end of different passages varied from 1.45 ± 0.04 million/cm^3 to 2.32 ± 0.29 million/cm^3. As for the test samples, cell concentration varied from 1.03 ± 0.03 million/cm^3 at passage 1 to 1.60 ± 0.05 million/cm^3 at passage 7. Cell growth rate in the test samples during the first six passages was 1.18–1.80 times lower than that in the control.

The use of the supplement had no effect on the pH of the medium during cultivation. The pH values changed identically in all the samples, being 6.84–7.11 at the beginning of the passage and decreasing to 6.34–6.48 after 48 hours.

Tests of specialised Sheff-Vax PF ACF supplement (No. 2) showed that cell concentration 48 hours after seeding in the control samples with constant serum content varied from 2.74 ± 0.08 to 1.80 ± 0.10 million/cm^3, and in the last passage 7 it was 2.24 ± 0.18 million/cm^3. Cell growth rates at different passages were in the range of 2.83 to 5.10 (Fig. 3, 4). Cell concentration at the end of passages in the control samples with reduced serum percentage in the nutrient medium and in the test samples with the specialised supplement was the same, but by passage 7 it declined by a factor of 1.4–1.9 when serum level was reduced to 0.075%. Cell growth rates in control 2 and test samples were also the same, namely 2.2–3.1 depending on the passage.

The use of the supplement had no effect on the pH of the medium during cultivation.

Tests of specialised Sheff-Vax Plus ACF supplement (No. 3) showed that cell concentration at passage 7 in the test samples with the medium almost free from serum was 1.0 ± 0.2 million/cm^3, in control 1 – 2.2 ± 0.2 million/cm^3, in control 2 – 1.20 ± 0.06 million/cm^3. Cell growth rate was 2.33 ± 0.03 in the test samples, and 3.57 ± 0.64 and 2.00 ± 0.53 in two control samples (Fig. 5, 6). Cell concentration in the control with constant serum content 48 hours after seeding at different passages was within the range of 2.06–2.38 million/cm^3; cell concentration in the samples with reduced serum content decreased from 2.32 ± 0.18 to 1.20 ± 0.06 million/cm^3 as the passage number increased. Growth rate in the controls with constant serum content was 3.4–5.0; growth rate in the control with reduced serum content was 3.33–4.00 at the first five passages and then decreased to 2.0 by passage 7. Cell growth rate in the test medium containing supplement 3 decreased by a factor of 1.6–1.8 after passage 4.

The use of this supplement had no effect on the pH of the medium during cultivation.

During FMDV reproduction in the cells, it was found that the concentration of 146+75S immunogenic components in the control samples with constant serum content

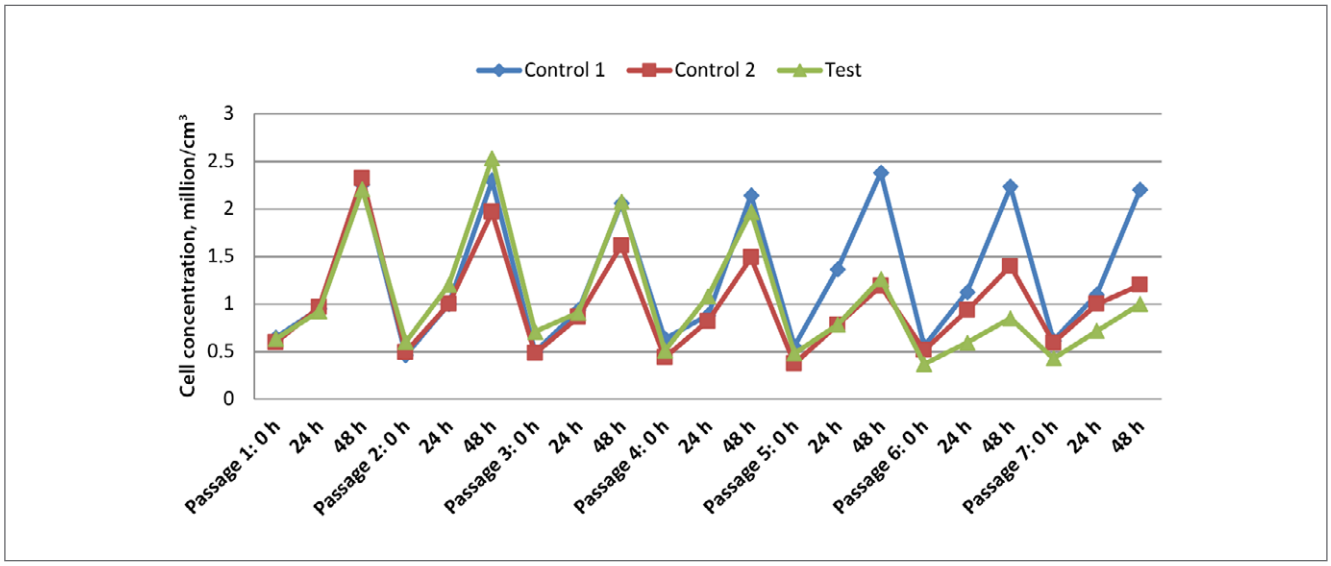

Fig. 5. BHK-21/SUSP/ARRIAH cell concentration dynamics in the presence of specialised Sheff-Vax Plus ACF supplement (No. 3)

Рис. 5. Динамика изменений концентрации клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax Plus ACF (№ 3)

was 1.57, 1.87, 1.60 times lower than in the control samples with reduced serum percentage, and 2.4, 2.3, 2.4 times lower than in the test samples (see Table). Differences were found to be significant ($p < 0.05$).

To study cell morphology, passage 7 suspension was seeded to 50 cm^3 flasks at a concentration of 100 thousand cells/cm^3; cytochemical tests were carried out at passage 8. Cells grown with supplement 1 demonstrated partial adhesion. Sedimented cells had normal morphology and multiple dynamic protrusions indicative of normal physiological activity (Fig. 7).

Partial sedimentation without adhesion was observed in the cells adapted to supplement 2 at passage 8. From the very beginning of cultivation, cell population aggregation was observed that reached its maximum on day 2. No culture proliferation was observed (Fig. 8A).

When supplement 3 was used (Fig. 8B), induced cell aggregation occurred and large colonies (up to 100 cells) were formed. Aggregated cells had irregular spherical shape and showed no signs of trophic activity, i.e. the cells did not divide but merely survived.

The serum-containing control (Fig. 7A) showed 100% confluence; the cells demonstrated 60–80% adhesion and had adaptive traits of a monolayer culture, some of them became spindle-shaped.

CONCLUSION

BHK-21/SUSP/ARRIAH cell line was adapted to specialised Sheff-Vax ACF supplements (Kerry, Inc., Ireland). By passage 7, cell concentration and growth rate in the presence of Sheff-Vax Plus PF ACF (supplement 1) were 40–60% higher than with other supplements. In the presence of supplement 1, cell concentration and growth rate in the test and control samples were equal: 1.6 ± 0.2 million/cm^3, 1.90 ± 0.18 and 2.0 ± 0.2 million/cm^3, respectively; differences were insignificant. Other supplements provided worse performance with respect to cell growth as compared to the control samples – cell concentration and growth rate were 2.0–2.2 times lower.

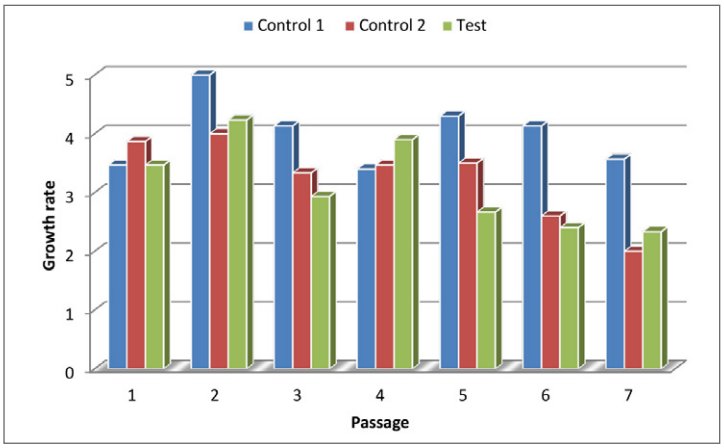

Fig. 6. BHK-21/SUSP/ARRIAH cell growth rate dynamics in the presence of specialised Sheff-Vax Plus ACF supplement (No. 3)

Рис. 6. Динамика изменений кратности прироста клеток BHK-21/SUSP/ARRIAH при использовании специализированной добавки Sheff-Vax Plus ACF (№ 3)

Table
FMDV reproduction in cells grown using specialised Sheff-Vax ACF supplements

Таблица
Репродукция вируса ящура в клетках, выращенных в присутствии специализированных добавок Sheff-Vax ACF

Supplement	146+75S concentration, μg/cm^3 (calculated for 1.0×10^6 cells/cm^3)		
	Control 1	Control 2	Test
Sheff-Vax Plus PF ACF (No. 1)	0.58 ± 0.09	0.91 ± 0.10	1.40 ± 0.10
Sheff-Vax PF ACF (No. 2)	0.47 ± 0.07	0.88 ± 0.08	1.08 ± 0.09
Sheff-Vax Plus ACF (No. 3)	0.50 ± 0.05	0.80 ± 0.08	1.20 ± 0.09
$p < 0.05$			

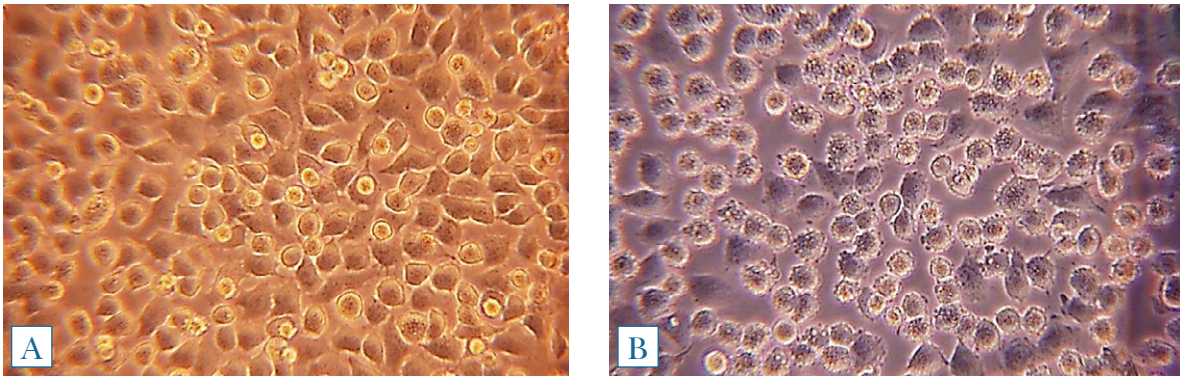

Fig. 7. BHK-21/SUSP/ARRIAH cells adapted to specialised Sheff-Vax Plus PF ACF supplement (No. 1):
A – cells grown in the presence of serum (control),
B – cells grown in the presence of supplement 1 (test)

Рис. 7. Клетки BHK-21/SUSP/ARRIAH, адаптированные к специализированной добавке Sheff-Vax Plus PF ACF (№ 1):
А – клетки, выращенные в присутствии сыворотки (контроль),
В – клетки, выращенные в присутствии добавки № 1 (опыт)

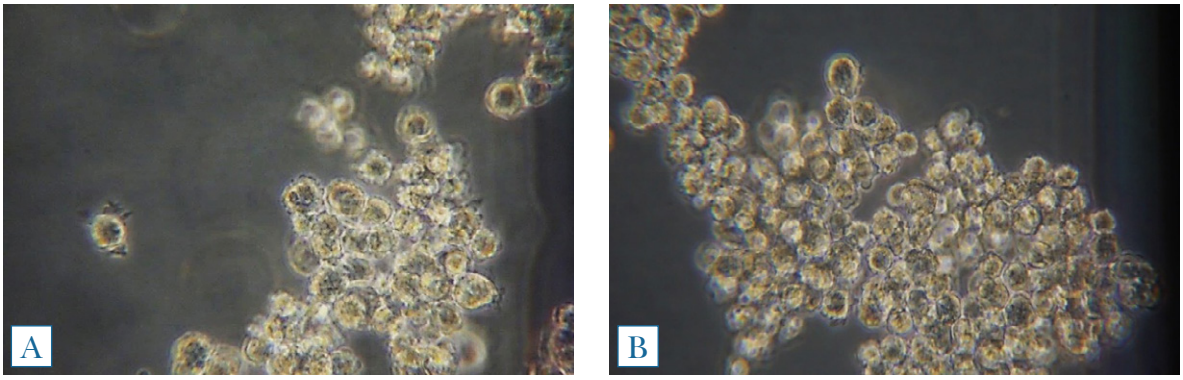

Fig. 8. BHK-21/SUSP/ARRIAH cells adapted to specialised supplements:
A – Sheff-Vax PF ACF (No. 2) and B – Sheff-Vax Plus ACF (No. 3)

Рис. 8. Клетки BHK-21/SUSP/ARRIAH, адаптированные к специализированным добавкам:
А – Sheff-Vax PF ACF (№ 2) и В – Sheff-Vax Plus ACF (№ 3)

No differences were found with respect to changes in pH.

During FMDV reproduction in the said cells, it was found that 146+75S component concentration in the test samples containing 1 million cells was 2.4, 2.3, 2.4 times higher compared to control 1, and 1.54, 1.23, 1.50 times higher compared to control 2 (differences were significant, $p < 0.05$).

Cells grown with the use of Sheff-Vax Plus PF ACF (supplement 1) had normal morphology and multiple dynamic protrusions. By passage 7, cell growth rate and concentration became equal in the suspension of the control and test samples. The number of FMDV immunogenic components in the samples containing supplement 1 was 20–30% higher than in the cells grown with supplements 2 and 3.

Concentration and growth rate of BHK-21/SUSP/ARRIAH cells cultivated with the use of Sheff-Vax ACF supplements were found to be lower than those in the control samples containing serum and blood protein hydrolysate. However, the virus yield from 1 million cells was higher in the cells grown using the specialised supplements.

Thus, serum-free Sheff-Vax ACF supplements (Kerry, Inc.) are suitable for BHK-21/SUSP/ARRIAH cell cultivation and FMDV reproduction. Sheff-Vax Plus PF ACF supplement (No. 1) provided higher results with respect to FMDV immunogenic component concentration.

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