GE Healthcare

Serum alternatives to fetal bovine serum in cell culture

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Introduction and objectives

Serum is often a necessary component of cell culture. Fetal bovine serum (FBS) has long been the first serum of choice for researchers. Although FBS performs well, there are circumstances wherein FBS replacements might offer advantages, for example, with regard to cost of sera, variability in supply, lot-to-lot variability in composition, and performance with specific cell types. This study examines the performance of FBS and seven serum alternatives with six cell lines.

Key concepts and findings

- Comparisons were made with the FBS control condition as a base standard, using a ratio of cell counts.
- Available FBS replacements are shown to work with six cell lines.
- The FBS replacements have advantages over FBS and provided equivalent or better cell growth compared with FBS (results are cell-line dependent).

Methodology

The six cell lines and eight serum types used here are listed in Tables 1 and 2. All cultures were grown in T-25 cell culture flasks in 10 mL of corresponding media supplemented with 10% serum. Control FBS was prepared by pooling many lots of FBS. FetalClone™ sera are blends of FBS and specially processed calf serum formulated to reproduce the composition of FBS. FetalClone I, II, and III are optimized for hybridoma, CHO, and fibroblast cells, respectively. Iron-Supplemented Calf Serum is produced from formula-fed veal animal serum supplemented with physiological levels of iron and contains high levels of transferrin. Both US and New Zealand Cosmic Calf™ sera are based on Iron-Supplemented Calf Serum with additional growth promoting factors. Bovine Growth Serum is also based on Iron-Supplemented Calf serum with additional trace elements, vitamins, and growth factors.

All conditions (except control FBS of which one lot was used, and supplementation of MRC-5 and AIF cells using Cosmic Calf, New Zealand Origin where two lots were used) consisted of three serum lots to test lot-to-lot consistency. Flasks were seeded at 3000–5000 cells/cm^2, incubated in 5% CO_2/95% air at 37°C, and checked daily for confluency. When any culture reached confluency, all cultures were trypsinized and counted. Cell counts were normalized to the FBS control as percentages such that the FBS control is always 1.0 or 100%. Conditions that produced more cells than the control have values greater than 1.0.

It was necessary to define a ratio at which condition performance was comparable with or better than control FBS. A value of 0.90 or 90% was chosen to accommodate experimental variations in harvesting and counting.

Table 1. Cell lines used

Cell line	Description	Source No.	Culture medium
MRC-5	Human lung fibroblasts	ATCC CCL-17	HyClone™ DMEM-High Glucose
BHK-21	Baby hamster kidney fibroblasts	ATCC CCL-10	HyClone MEM
VERO	African green monkey kidney fibroblasts	ATCC CCL-81	HyClone MEM
CHO-K1	Chinese hamster ovary epithelial cells	ATCC CCL-61	HyClone Ham's F-12
AIF	Hybridoma: mouse, lymphocyte-like myeloma NS1	ATCC TIB-131	HyClone DMEM-High Glucose
NS0	Myeloma	ECACC 85110503	HyClone RPMI-1640

Table 2. Sera tested

Sera
HyClone Fetal Bovine Serum (FBS), control
HyClone FetalClone I
HyClone FetalClone II
HyClone FetalClone III
HyClone Cosmic Calf Serum, New Zealand Origin
HyClone Iron Supplemented Calf Serum
HyClone Cosmic Calf Serum, US Origin
HyClone Bovine Growth Serum

Results and discussion

Results were cell line-dependent with certain FBS replacements proving to be more or, sometimes, less suitable for specific cell lines. In nearly all cases, cell growth in at least one of the FBS replacements was equal to or greater than cell growth in FBS. This finding indicates that researchers have viable FBS alternatives for replacements in their cell cultures.

MRC-5 cells grew more rapidly, and thus to higher yields, in FetalClone III and Bovine Growth Serum than in FBS (Fig 1). In comparison with cell yields in FBS, Vero cell yields were at least as high in FetalClone II, FetalClone III, New Zealand Cosmic Calf, IronSupplemented Calf, US Cosmic Calf, and Bovine Growth Serum (Fig 2). The rate of BHK-21 cell growth was about the same in FBS, FetalClone II, Fetal Clone III, and U.S. Cosmic Calf Serum, while these cell growth was more rapid in Bovine Growth Serum (Fig 3).

FetalClone II is optimized for CHO cells, as are the Cosmic Calf sera. Supplementation with FetalClone II, FetalClone III, New Zealand Cosmic Calf and US Cosmic Calf each resulted in higher yields of CHO cells than did FBS (Fig 4). Bovine Growth Serum performed comparably to FBS.

AIF cells were used as a model for conventional hybridoma cell lines. All sera tested supported hybridoma cell growth rates equal to or higher than with FBS (Fig 5). However, for many hybridoma applications, the lower IgG levels in FBS and FetalClone I make these the preferred sera for monoclonal antibody production. The levels of some components of special interest are listed in Table 3.

NS0 cultures in FetalClones I, II, III, New Zealand Cosmic Calf, Iron Supplemented Calf, and US Cosmic Calf serum showed growth equal to or better than that of growth in FBS (Fig 6).

Table 3. Levels of special interest components in the seven serum products evaluated

	IgG (mg/mL)	Total protein (g/dL)	Iron (µg/dL)	Transferrin (mg/dL)	Cholesterol (mg %)
Fetal Bovine Serum, control	0.190	3.9	175.2	188.1	35
FetalClone I	0.104	4.1	558.0	556.0	101
FetalClone II	0.096	4.1	557.0	559.0	111
FetalClone III	0.100	3.6	558.0	480.0	71
Cosmic Calf Serum, New Zealand Origin	18.70	7.5	269.0	240.0	Not tested
Iron Supplemented Calf Serum	13.63	7.0	630.0	647.2	118
Cosmic Calf Serum, US Origin	10.20	6.6	640.0	634.0	Not tested

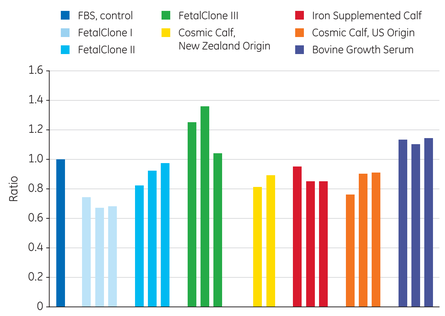

Fig 1. MRC-5 cells cultured in DMEM-High Glucose supplemented with 10% serum.

Fig 2. Vero cells cultured in MEM supplemented with 10% serum.

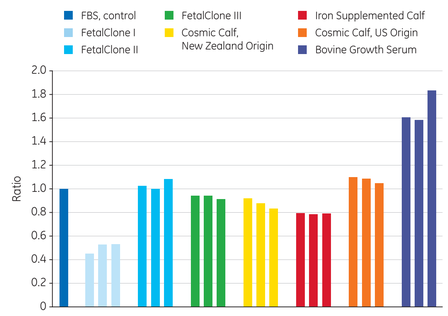

Fig 3. BHK-21 cells cultured in MEM supplemented with 10% serum.

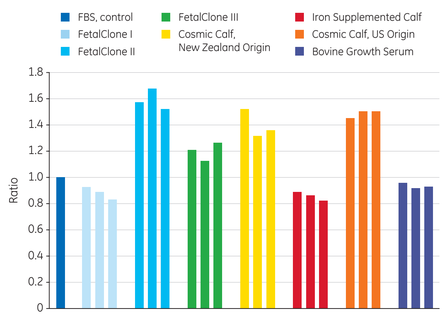

Fig 4. CHO-K1 cells cultured in Ham's F-12 supplemented with 10% serum.

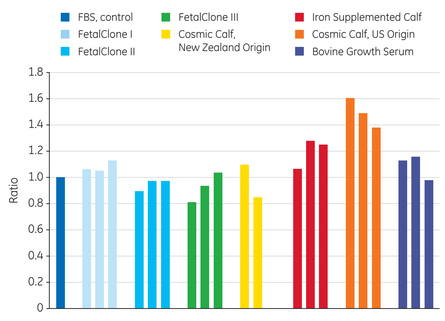

Fig 5. AIF cells cultured in DMEM-High Glucose supplemented with 10% serum.

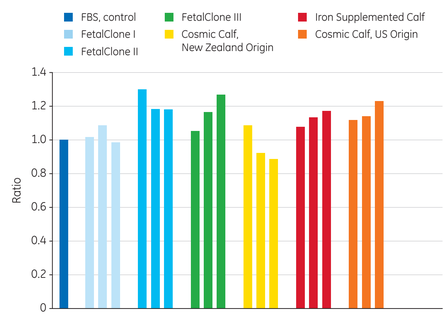

Fig 6. NS0 cells cultured in RPMI-1640 supplemented with 10% serum.

Study conclusions

This study has shown that multiple sera are available as potential replacements for FBS in cell culture. A variety of mammalian cell types (fibroblasts, hybridoma, myeloma) were used in the study, and each type was shown to have a potential FBS replacement in at least one bovine calf-based serum. Some advantages of the tested calf-based sera compared with FBS are lower cost, higher availability, and perhaps more consistent component levels due to the methods used in the veal industry. Animal age at time of slaughter, stress on the animals, breed, and diet are factors that can contribute to the consistent component levels in calf sera compared with the same composition in fetal bovine serum.

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