Reducing serum supplementation costs with HyQ™-RS media

To reduce the need for fetal bovine serum (FBS) in cell culture applications, there are several viable alternatives available. These studies compare cell growth in HyQ-RS media supplemented with reduced concentrations of FBS with growth in classical versions of the same media supplemented with traditional FBS concentrations. In addition, the performance of bovine growth serum (BGS) as FBS replacement was investigated.

Introduction

With increasing demands for FBS while supplies continuing to fluctuate, researchers are looking for alternative cell culture nutrient solutions. There are several options available, such as using FBS replacements or serum-free media, or to reduce the required supplementation with FBS in basal media.

The first two options are gaining popularity, and GE Healthcare offers several FBS replacements, such as HyClone™ Bovine Growth Serum and HyClone FetalClone™ engineered serum products, as well as an extensive selection of cell-line specific serum-free media. However, reducing concentration of FBS in the basal medium can also be an effective alternative. In many cases, it is possible to reduce the concentration of FBS used in standard basal to as low as 2% from the standard of 10%. Further reduction of FBS concentrations can be made by using an enhanced basal medium, such as the HyClone HyQ-RS reduced serum media.

The HyQ-RS media are based on classical formulations, but the formulations are enhanced with antioxidants and other nutritional factors to compensate for the lower levels of critical serum factors as FBS concentrations are decreased. HyQ-RS media enable cost-efficient cultivation by allowing extended use of a selected serum lot. Compared with FBS, HyQ-RS media decrease the variability in culture caused by serum lot-to-lot variation. In addition, HyQ-RS media ease regulatory concerns by reducing the amount of serum in the culture medium.

This application note compares classical media with HyQ-RS media. Three commonly used cell lines were cultured with little or no adaptation to culture conditions. The results show equivalent cell growth, even when HyQ-RS media are supplemented with serum concentrations as low as 2% FBS.

Materials and methods

Stock cells were maintained in classical medium supplemented with 10% FBS. Cells were centrifuged and re-suspended in PBS to remove all traces of serum. Thereafter, the cells were directly seeded into the conditions studied. All cell cultures were maintained at 37° C with 5% CO_2.

Growth promotion studies

Cell lines and culture conditions used in basic growth promotion studies are listed in Table 1.

Table 1. Cell lines, basal media, and serum supplementation used in basic growth promotion studies

Cell line	ATCC Cat. #	Description	Culture medium	Serum supplement
Vero	ATCC CCL 81	African green monkey	MEM/EBSS	10% or 2% FBS
			DMEM/ High Glucose	10% or 2% FBS
			HyQ MEM-RS	2% FBS
			HyQ DMEM-RS	2% FBS
Sp2/0	ATCC CRL8006	Mouse hybridoma	DMEM/ High Glucose	10% or 2% FBS
			HyQ DMEM-RS	2% FBS

The studies were varied depending on the characteristics of the cell lines: attaching or suspension.

Adherent Vero cells were seeded at 1.0×10^5 cells per flask into duplicate T-25 flasks in a volume of 6 mL. Cells were examined daily for confluence and morphology. Cells were trypsinized and counts from each flask were taken daily and averaged until the control flasks reached 100% confluence.

Suspension Sp2/0 cells were seeded at 7.5×10^5 cells per flask into duplicate T-75 flasks in a volume of 25 mL. Cells were examined and counts from each flask were taken daily and averaged until viability began to decline.

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Growth promotion study including Caco-2 cells

Cell line and culture conditions used in the Caco-2 cell growth promotion study are listed in Table 2. Cells were seeded at 5.0×10^5 cells per flask into duplicate T-25 flasks in 6 mL volume. Cells were examined daily for confluence and morphology. Cells were detached with trypsin and counts from each flask were taken daily and averaged until the control flasks reached 100% confluence.

Table 2. Cell line, basal media, and supplementation used in Caco-2 cell growth promotion study

Cell line	ATCC Cat. #	Description	Culture medium	Supplement
Caco-2	ATCC HTB 37	Human adenocarcinoma, colon	MEM/EBSS HyQ MEM-RS	20% FBS + NEAA* + sodium pyruvate 0%, 8%, 10%, 12%, or 14% FBS + NEAA*

* Non-essential amino acid

Growth promotion study including supplementation with BGS

Cell line and culture conditions use in the BGS growth promotion study are listed in Table 3. Vero cells were seeded at 1.0×10^6 cells per flask in duplicate T-25 flasks in a volume of 6 mL. Cells were examined daily for confluence and morphology. Cells were trypsinized and counts from each flask were taken daily and averaged until the control flasks reached 100% confluence.

Table 3. Cell lines, basal media, and supplementation used in BGS growth promotion study

Cell line	ATCC Cat. #	Description	Culture medium	Supplement
Vero	ATCC CCL 81	African green monkey	DMEM/ High Glucose HyQ DMEM-RS	10% BGS 2% FBS, 2% BGS, or 4% BGS

Long-term passage study

Cell line and culture conditions use in the long term passage study are listed in Table 4. Caco-2 cells were seeded at 1.5×10^6 cells per flask into duplicate T-75 flasks in a 25 mL volume. Cells were examined daily for confluence and cell morphology. Cells were counted and passed every 3 to 4 when the control reached 100% confluence for a total of 4 passages.

Table 4. Cell line, basal media, and supplementation used in long-term passage study

Cell line	ATCC Cat. #	Description	Culture medium	Supplement
Caco-2	ATCC HTB 37	Human adenocarcinoma, colon	MEM/EBSS HyQ MEM-RS	20% FBS + NEAA* + sodium pyruvate 10% FBS + NEAA*

* Non-essential fatty acid

Results

Growth promotion

Results from basic growth promotion studies including Vero and Sp2/0 cells are shown in Figures 1–3. As shown, HyQ-RS media supplemented with reduced concentrations (2%) of FBS perform well when compared with classical versions of the same media supplemented with traditional concentrations (10%) of FBS. The additional components included in the HyQ-RS formulations support cell growth with reduced concentrations of FBS to levels comparable with growth in classical medium supplemented with 10% FBS. For Vero cells, the growth was even improved in HyQ-RS media with 2% FBS supplementation compared with in classical media with 10% FBS supplementation.

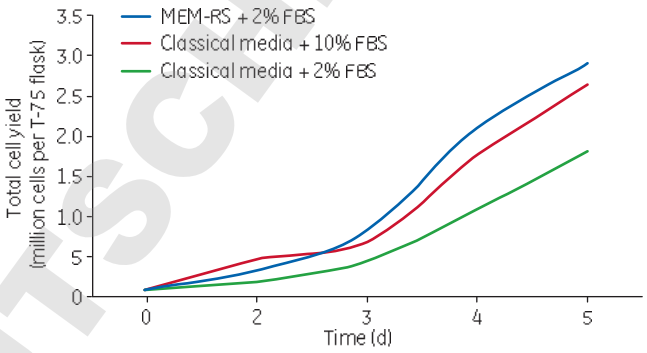


Fig 1. Vero cell growth in classical MEM supplemented with 10% or 2% FBS as well as in MEM-RS supplemented with 2% FBS.

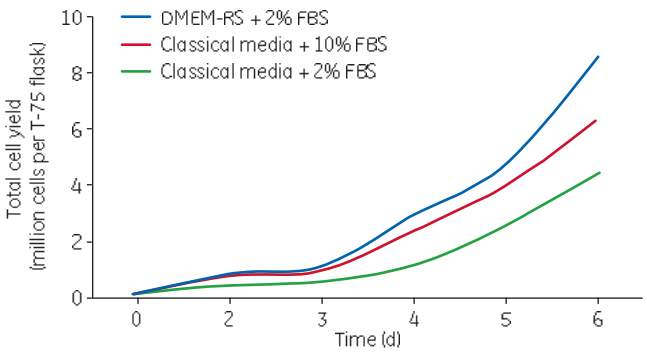


Fig 2. Vero cell growth in classical DMEM supplemented with 10% or 2% FBS as well as in DMEM-RS supplemented with 2% FBS.

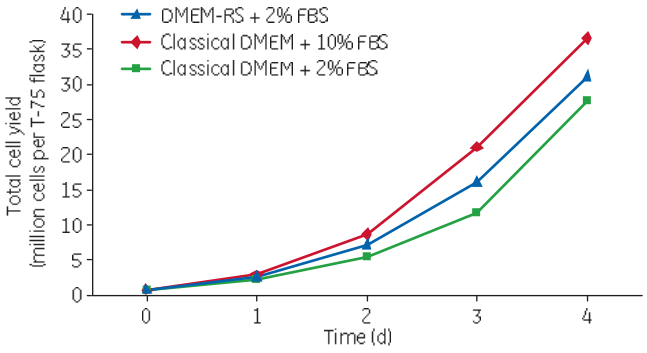


Fig 3. Sp2/0 cell growth in classical DMEM supplemented with 10% or 2% FBS, as well as in DMEM-RS supplemented with 2% FBS.

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Growth promotion using BGS supplementation

Growth in HyQ DMEM-RS medium supplemented the FBS replacement BGS was investigated. Vero cells were grown in HyQ DMEM-RS medium supplemented with 2% and 4% BGS. Vero cells grown in classical DMEM supplemented with 10% BGS or in HyQ DMEM-RS medium supplemented with 2% FBS were used as controls. Results show comparable growth in all conditions studied (Fig 4).

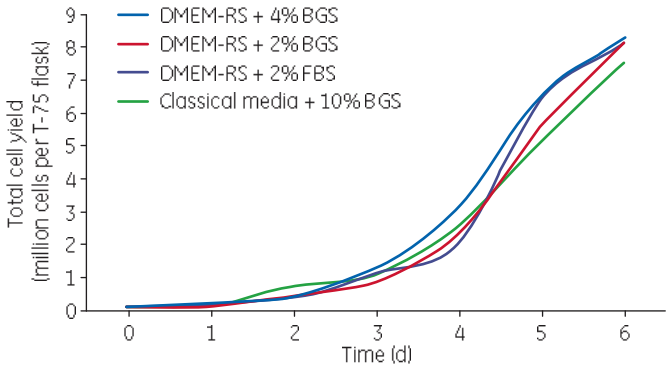


Fig 4. Vero cells growth in classical DMEM supplemented with 10% FBS and well as in DMEM-RS supplemented with 2% FBS, 4% BGS, or 2% BGS.

Growth promotion of Caco-2 cells

Caco-2 cells are generally grown in medium supplemented with 20% FBS. A study was initiated to investigate the growth of Caco-2 cells in HyQ MEM-RS medium supplemented with FBS concentrations of 0%, 8%, 10%, 12%, and 14% and non-essential amino acids (NEAA). These conditions were compared with a control culture with classical MEM/EBSS supplemented with NEAA, sodium pyruvate, and 0% and 20% FBS (Fig 5). The condition containing 0% FBS did not show cell attachment until day 4, hence, these cultures were terminated.

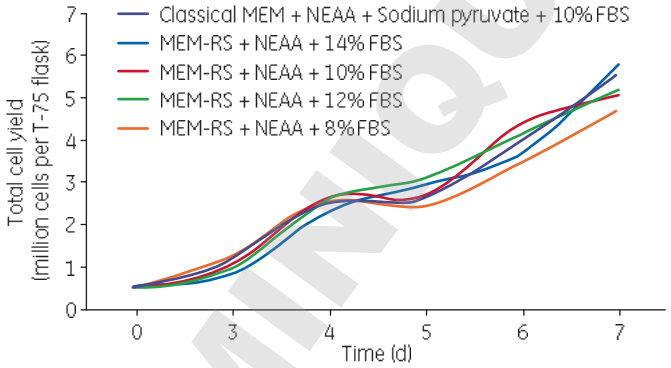


Fig 5. Caco-2 cell growth in classical MEM supplemented with 20% FBS, as well as in MEM-RS supplemented with 14%, 10%, or 8% FBS. Cultures were supplemented with NEAA and sodium pyruvate as required.

Long-term passage

A long-term passage study was performed using Caco-2 cells grown in classical MEM supplemented with NEAA, sodium pyruvate, and 20% FBS (control) and in HyQ MEM-RS supplemented with NEAA and 10% FBS. Results show comparable growth in HyQ MEM-RS cultures to control cultures.

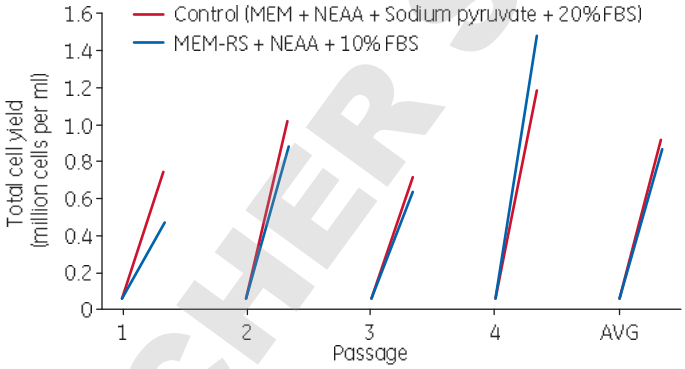


Fig 6. Multi-passage study using Caco-2 cells grown in classical MEM supplemented with 20% FBS and in MEM-RS supplemented with 10% FBS. Cultures were supplemented with NEAA and sodium pyruvate as required.

Conclusion

The results show comparable or improved cell growth in HyQ-RS media with reduced serum supplementation to growth in known and trusted classical version of the media for cell lines and conditions tested. Hence, we conclude that HyQ-RS media can reduce laboratory costs and other concerns with serum use. The cost savings of serum supplementation can be further reduced when combining a HyQ-RS medium with an FBS replacement such as BGS. Data indicates that cells grow well in HyQ-RS medium supplemented with as little as 2% BGS. The results also reveal that little or no adaptation is required to grow the studied cells in HyQ-RS media. However, some adaptation can be necessary for other cell lines, such as MRC-5 cells, not evaluated in this study.

Ordering information

Product	Product code
HyClone DMEM/High Glucose	SH30022
HyClone MEM/EBSS	SH30024
HyClone HyQ MEM-RS	SH30564
HyClone HyQ DMEM-RS	SH30565
HyClone Bovine Growth Serum	SH30541
HyClone Non-Essential Amino Acids	SH30238
HyClone Dulbecco's Phosphate Buffered Saline	SH30028

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