Serum stability at refrigeration temperatures

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Serum stability at refrigeration temperatures

For serum to retain stability during long-term storage, serum should be stored frozen. However, thawed serum for short-term storage is commonly refrigerated. This application note shows the suitability of serum for use as basal medium supplement in cell cultures applications after storage in refrigerator (2°C to 8°C) for up to 26 weeks.

Introduction

Fetal bovine serum (FBS) is most stable when stored frozen at -10°C or lower, and we have validated a five year shelf life for FBS stored at this temperature. From a practical standpoint, researchers frequently store unused portions of thawed FBS at refrigeration temperatures between 2°C to 8°C. Technical Support is often asked for a recommended time period for which FBS can be stored at this temperature. Historically, our conservative recommendation has been to store the serum no longer than six to eight weeks at this temperature, but from informal studies and customer input, we perceived that most cell lines might perform suitably using serum stored at 2°C to 8°C for longer periods of time. The data presented here corroborate this hypothesis that FBS stored at 2°C to 8°C for time periods longer than eight weeks remains suitable for many cell culture applications.

Methods

Serum from two lots of Defined Fetal Bovine Serum stored at 2°C to 8°C for up to 26 weeks was used in this study. The ability of this serum to support growth was evaluated using Vero, CHO-K1, MRC-5, FOX-NY, and Sp2/0-Ag14 cells. Further information on these cell lines, including the basal medium used for each, can be found in Table 1. Basal media were supplemented to 10% with the serum to be evaluated and the cells were grown at 37°C in an atmosphere of 5% CO_2. The attaching cell lines were seeded at a density of 5×10^4 cells/flask in 25 cm² T- flasks, and counted just prior to confluence. The suspension cell lines were seeded at 2.5×10^4 cells/mL in 25 cm² T- flasks and counted daily for five days. Each condition was set up in triplicate. Freshly thawed serum of the same serum lot was used as a control for this study.

Results and discussion

Figure 1 shows the results of the attaching cell lines (Vero, CHO-K1, and MRC-5). These results indicate little, if any, significant difference in cell growth between using the freshly thawed serum (week 0) and the serum stored at 2°C to 8°C for 24 weeks with any of the attaching cell lines.

Table 1. Cell lines used in the study

Cell line	Origin	Morphology	Tissue	Medium used
VERO	African green monkey	Fibroblast-like	Kidney	MEM/EBSS
CHO-K1	Chinese hamster	Epithelial-like	Ovary	HAMS F-12
MRC-5	Human	Fibroblast-like	Lung	DME/High
Sp2/0-Ag14	Mouse	Suspension	Myeloma	DME/High
FOC-NY	Mouse	Suspension	Myeloma	DME/High

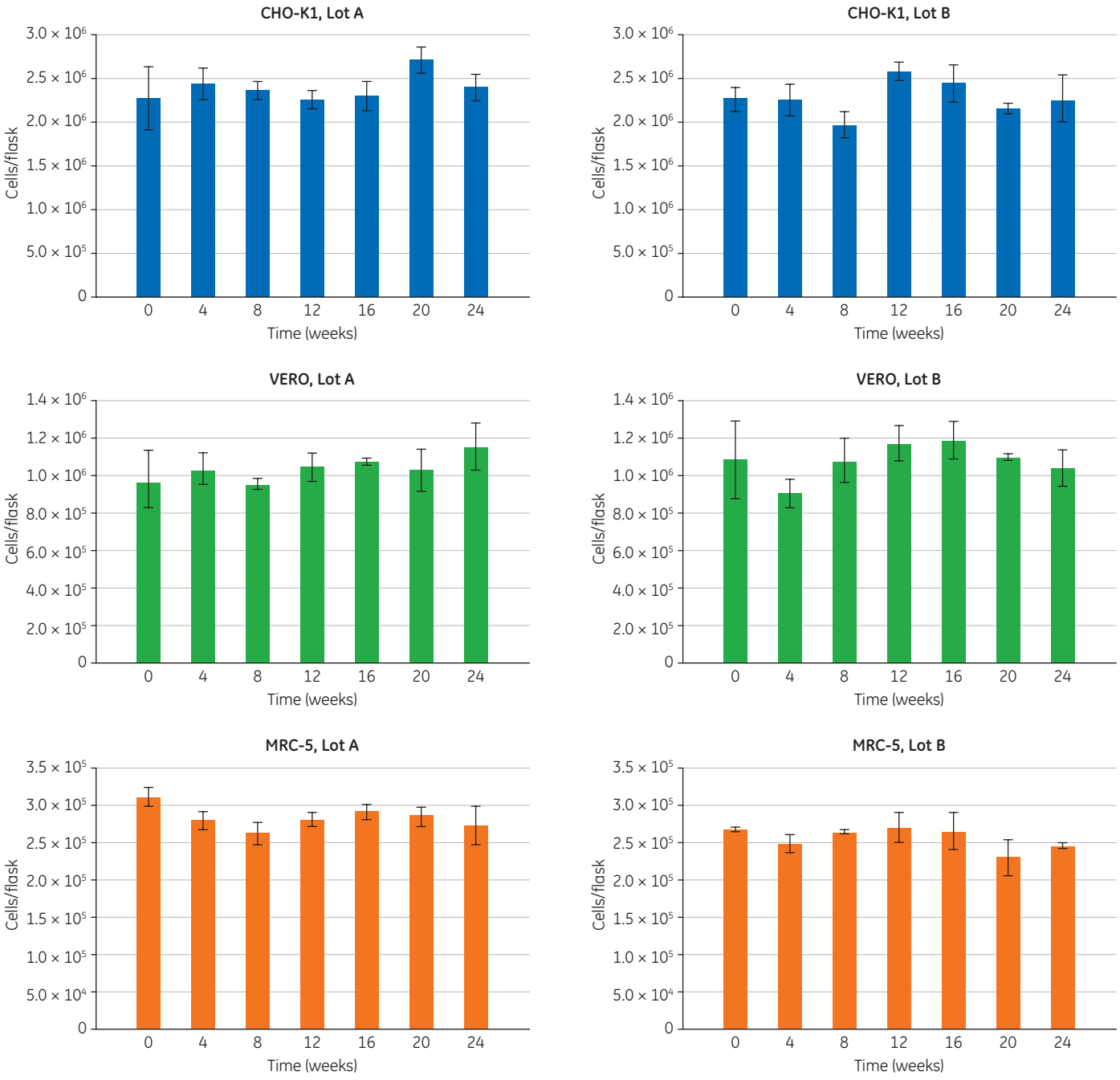

Fig 1. Cell counts of CHO-K1, Vero, and MRC-5 cells grown in basal medium with 10% serum stored for up to 24 weeks at 2°C to 8°C. Lot A and Lot B indicate the two separate serum lots used in the study. Error bars represent ± 1 standard deviation.

Figure 2 shows growth curves of the non-attaching cell lines (Sp2/0-Ag14 and FOX-NY). Comparison of the growth curves generated by culture using the freshly thawed serum (week 0) to the curves from using 26 week refrigerated serum also shows little, if any, difference in the growth patterns. Both serum lots produced similar results with the cell lines tested.

The data from all cell lines tested, using freshly thawed serum and serum stored for up to 6 months at 2°C to 8°C, show no significant differences in final cell numbers. From these data it seems reasonable to assume that serum stored for up to six months in cold room conditions (2°C to 8°C) properly provides for the needs of these cell lines and perhaps others. This study used five cell lines and measured only cell concentration. It would be presumptuous to assume that serum stored at 2°C to 8°C for six months is appropriate for all cell lines, all conditions, and all objectives. If long-term storage at 2°C to 8°C might be useful to you, we recommend testing such serum with your cells, under your conditions, and evaluating the performance based on your criteria.

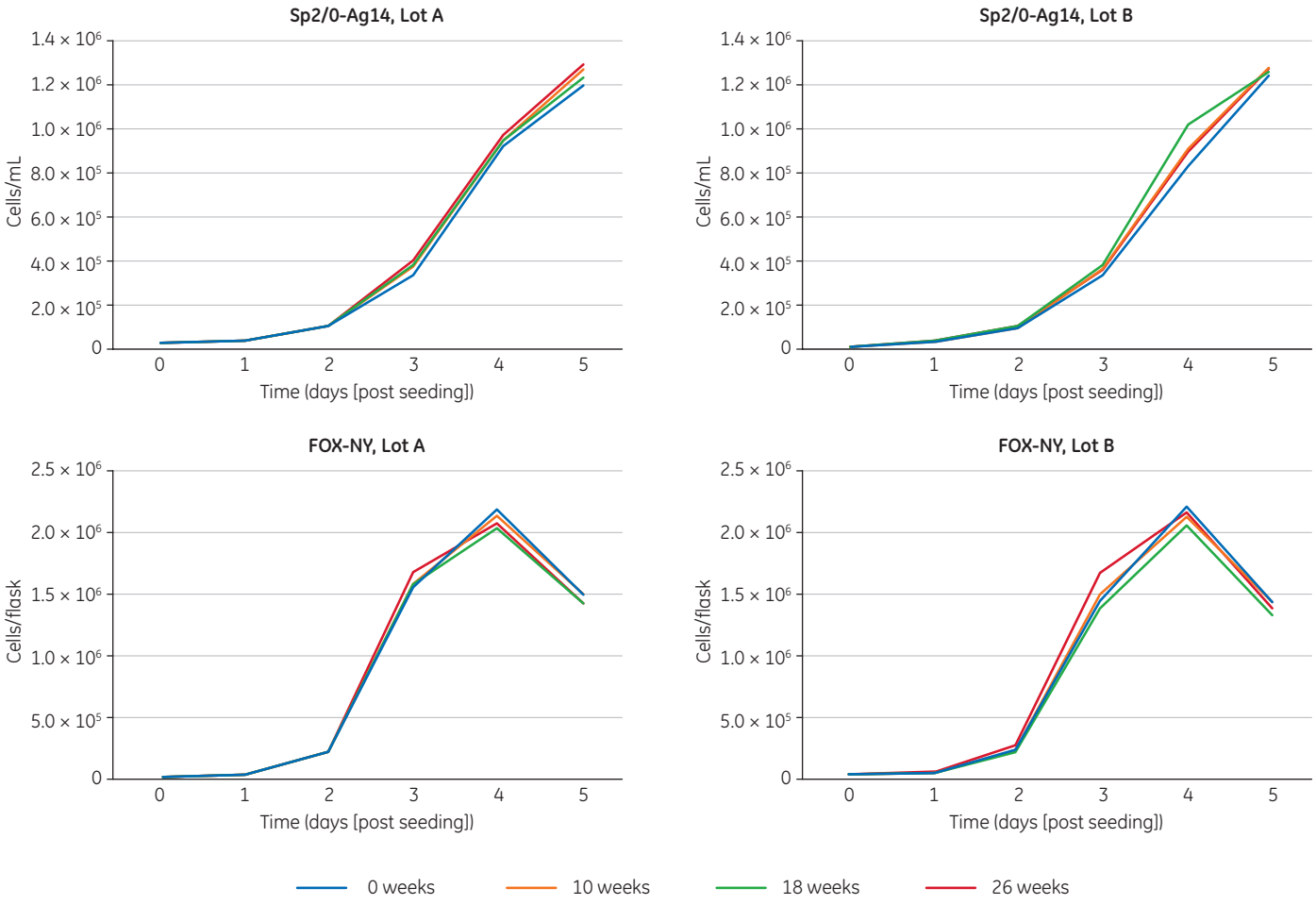

Fig 2. Growth curves of Sp2/0-Ag14 and FOX-NY cells grown in basal medium with 10% serum stored for up to 26 weeks at 2°C to 8°C. Lot A and lot B indicate the two separate serum lots used in this study.

Conclusion

This study shows little or no difference in growth of cells cultured in basal medium supplemented with either freshly thawed serum or serum stored at 2°C to 8°C for up to 26 weeks. Both adherent and suspension cells were included in the study. However, if refrigeration temperature for storage of serum for up to six month should be applied, it is recommended that the performance of the selected cell line in its specific application is tested with such serum.

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