Serum storage, handling, and thawing

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CY14861-26Jun20-AN

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This application note addresses common storage and handling questions and provides specific instructions and recommendations for storing and handling of HyClone™ serum products.

Introduction

Serum is a supplement used to enhance cell culture performance, and contains several growth promoting factors including hormones, proteins, amino acids, glucose, and vitamins. Depending on source origin, HyClone sera are processed in 1000 L, 2000 L, 2500 L, and 3000 L lot sizes. To ensure uniformity and consistency between bottles, each serum lot is filtered and collected in a pool before dispensing (true pool technology).

Long-term, serum should be stored at -10°C or lower. Here, we present results from studying the effects of repeated freezing and thawing on serum performance with various cell lines.

Methods

One lot of HyClone Cosmic Calf™ Serum and one lot of HyClone Characterized Fetal Bovine Serum (FBS) were selected for this study. Five bottles from each lot were marked 1× thru 5×. Bottle 1× was kept frozen, and bottles 2× thru 5× were thawed at room temperature under a fan. Each bottle was mixed every 20 to 30 min while being thawed.

Upon complete thaw, the bottles were immediately refrozen and left overnight at -20°C. The process was repeated for bottles 3× thru 5×, and again repeated for 4× and 5×, and finally, again repeated for 5×.

Growth study

Five cell lines were used to determine the ability of the conditioned serum to support growth. The cell lines and the basal media used are listed in Table 1. All media were supplemented using 10% of the test serum. Adherent cells were seeded in triplicate at cell densities of 1.0×10^4 cells/cm^2 in T-25 flasks and were counted upon reaching 90% confluence. Suspension cells were seeded in triplicate at cell densities of 8.0×10^3 cells/mL in T-25 flasks and were counted daily beginning on day 3 until cell viability dropped. All flasks were incubated at 37°C in a 5% CO_2 atmosphere.

Biochemical analysis

Samples from all serum bottles were sent for biochemical analysis to determine if a detectable change in commonly tested serum components could be determined.

Precipitation analysis

Samples were analyzed for precipitation by turbidimetry and by visual examination to determine if additional freezing cycles increase the amount of particulate matter.

Table 1. Cells and media used in this study

	Species	Morphology	Tissue	Medium used
VERO	African Green Monkey	Fibroblast-like	Kidney	DME, high glucose
CHO-K1	Chinese hamster	Epithelial-like	Ovary	HAMS F-12
MRC-5	Human	Fibroblast-like	Lung	DME, high glucose
Sp2/0-Ag14	Mouse	Suspension	Myeloma	DME, high glucose
FOX-NY	Mouse	Suspension	Myeloma	DME, high glucose

Results and discussion

Growth results are shown in Figures 1 to 5. In all cases, no significant trends in cell growth were observed. Results from biochemical analyses show no significant trends in the components measured (Table 2). Turbidimetric analyses indicate no detectable differences in the amount of particulate matter (Table 3). These results were confirmed by visual examination of the bottles.

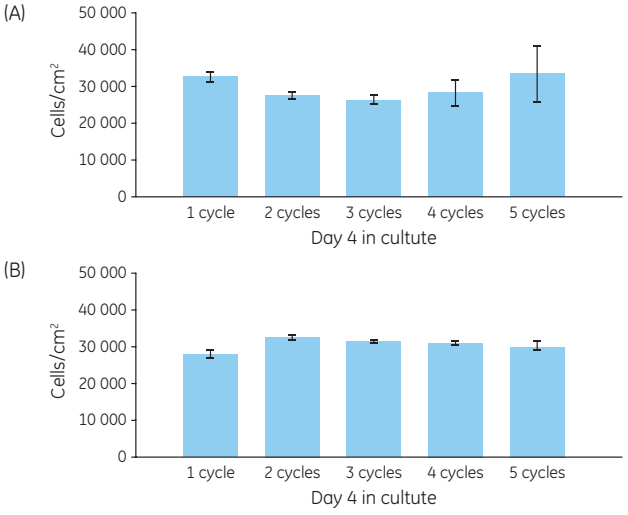

Fig 1. Growth of MRC-5 cells in DME, high glucose medium containing either 10% (A) Characterized FBS or (B) Cosmic Calf Serum, both subjected to multiple freeze/thaw cycles. Error bars indicate ± 1 standard deviation.

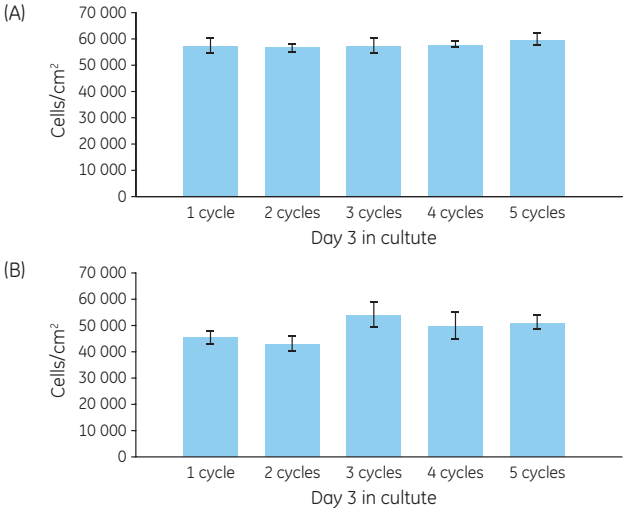

Fig 2. Growth of Vero cells in DME, high glucose medium either 10% (A) Characterized FBS or (B) Cosmic Calf Serum, both subjected to multiple freeze/thaw cycles. Error bars indicate ± 1 standard deviation.

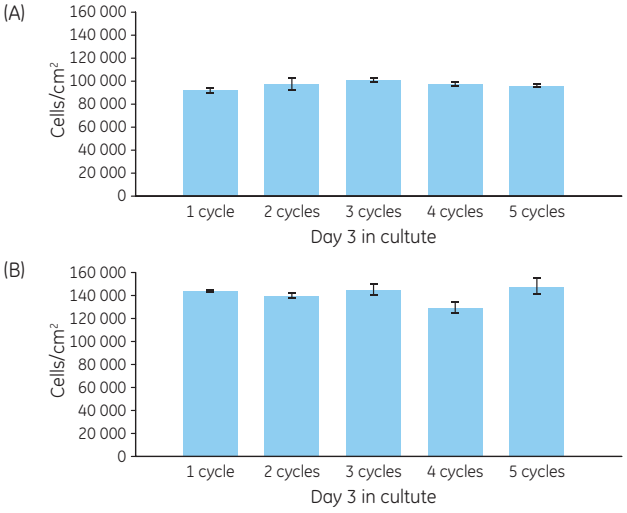

Fig 3. Growth of CHO-K1 cells in Ham's F12 medium containing either 10% (A) Characterized FBS or (B) Cosmic Calf Serum, both subjected to multiple freeze/thaw cycles. Error bars indicate ± 1 standard deviation.

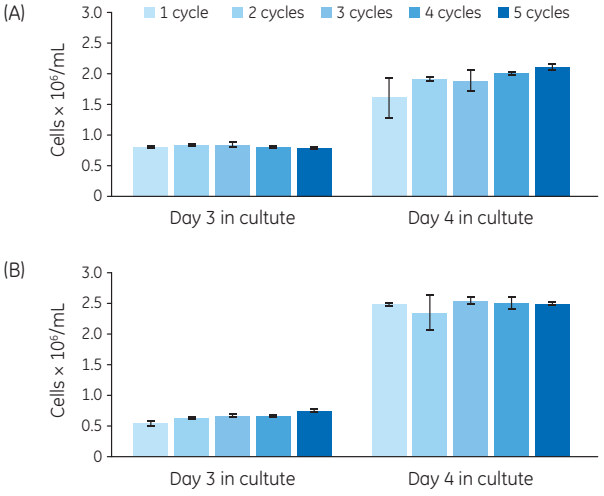

Fig 4. Growth of FOX-NY cells in DME, high glucose medium containing either 10% (A) Characterized FBS or (B) Cosmic Calf Serum, both subjected to multiple freeze/thaw cycles. Error bars indicate ± 1 standard deviation.

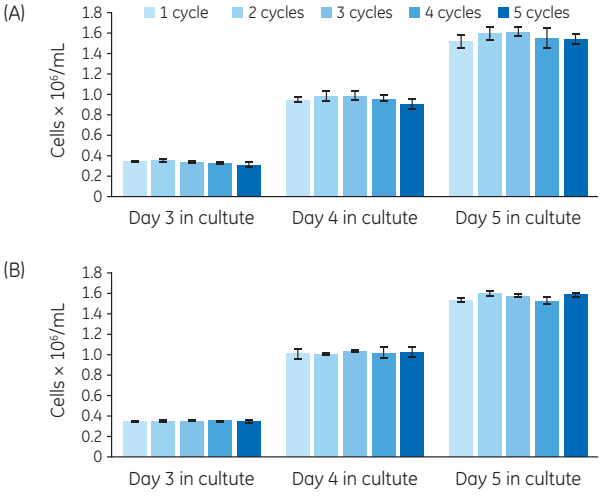

Fig 5. Growth of Sp2/0-Ag14 cells in DME, high glucose medium containing either 10% (A) Characterized FBS or (B) Cosmic Calf Serum, both subjected to multiple freeze/thaw cycles. Error bars indicate ± 1 standard deviation.

Table 2. Biochemical analyses of serum subjected to multiple freeze/thaw cycles

	Number of freeze/thaw cycles				
Characterized FBS	**1×**	**2×**	**3×**	**4×**	**5×**
Parathyroid hormone (PTH) (U/L)	48	39	36	46	39
T4 (µg/dL)	13.7	13.5	13.6	13.3	13.5
T3 (ng/dL)	95	98	98	95	97
Corticosterone (ng/dL)	< 20	24	< 20	27	< 20
Cortisol (µg/dL)	< 1	< 1	1.1	< 1	< 1
Progesterone (ng/dL)	< 10	< 10	< 10	< 10	< 10
Testosterone (ng/dL)	15	16	15	15	15
Insulin (µU/mL)	3	3.4	< 2.0	2.2	2.1
Sodium (mEq/L)	132	131	132	132	131
Potassium (mEq/L)	9.6	9.6	9.9	9.6	9.7
Chloride (mmol/L)	100	99	100	98	99
Glucose (mg/dL)	51	51	51	51	51
Blood urea nitrogen (BUN) (mg/dL)	14	14	13	13	13
Creatinine (mg/dL)	2.4	2.3	2.4	2.3	2.4
Calcium (mg/dL)	12.6	12.6	12.8	12.5	12.7
Magnesium (µg/mL)	3.1	3.1	3.1	3.1	3.1
Phosphorus (mg/dL)	9.7	9.6	9.6	9.5	9.7
Total protein (gm/dL)	3.2	3.2	3.2	3.2	3.2
Albumin (g/dL)	1.6	1.6	1.6	1.6	1.6
Cholesterol (mg/dL)	< 50	< 50	< 50	< 50	< 50
Bilirubin (mg/dL)	0.5	0.5	0.4	0.5	0.4
Alkaline phosphatase (U/L)	184	181	186	181	182
Lactate dehydrogenase (LDH) (U/L)	3223	3284	3166	3191	3005
Serum glutamate pyruvate transaminase (SGPT) (U/L)	33	36	35	35	37
Serum glutamic-oxaloacetic transaminase (SGOT) (U/L)	195	188	191	189	197
Cosmic Calf Serum	**1×**	**2×**	**3×**	**4×**	**5×**
PTH (U/L)	48	43	44	44	45
T4 (µg/dL)	8.2	8	8.6	6.5	8.6
T3 (ng/dL)	219	205	215	192	217
Corticosterone (ng/dL)	259	234	280	218	276
Cortisol (µg/dL)	2.4	2.6	1.9	1.4	2.1
Progesterone (ng/dL)	25	24	24	17	39
Testosterone (ng/dL)	5.9	5.1	6.1	4.6	5.1
Insulin (µU/mL)	2.9	2.9	2.1	< 2	4
Sodium (mEq/L)	141	141	142	140	140
Potassium (mEq/L)	5.6	5.6	5.7	5.6	5.6
Chloride (mmol/L)	101	104	101	101	101
Glucose (mg/dL)	89	89	89	89	89
BUN (mg/dL)	7	7	7	7	7
Creatinine (mg/dL)	1.3	1.3	1.3	1.3	1.2
Calcium (mg/dL)	10.4	10.2	10.2	10.2	10.2
Magnesium (µg/mL)	2.2	2.2	2.2	2.2	2.3
Phosphorus (mg/dL)	9.3	9.4	9.4	9.4	9.4
Total protein (gm/dL)	6.4	6.4	6.4	6.5	6.3
Albumin (g/dL)	2.8	2.8	2.8	2.8	2.8
Cholesterol (mg/dL)	101	101	100	101	101
Bilirubin (mg/dL)	0.3	0.3	0.3	0.2	0.2
Alkaline phosphatase (U/L)	205	206	208	205	206
LDH (U/L)	4398	4220	4202	4136	3955
SGPT (U/L)	27	30	28	27	28
SGOT (U/L)	105	106	106	104	105

Table 3. Biochemical analyses of serum subjected to multiple freeze/thaw cycles

Turbidimetric determinations expressed in nephelometric turbidity unit (NTU)	Number of freeze/thaw cycles				
	1×	2×	3×	4×	5×
Cosmic Calf Serum	34.6	35.5	36.3	35.4	35.3
Characterized FBS	10.5	10.3	10.3	10.2	9.97

Conclusion

Repeated freezing and thawing of serum

As no significant differences in growth performance, biochemical analysis data, or turbidity of serum subjected to five freeze/thaw cycles were detected, we feel confident in recommending that serum subjected to multiple freeze/thaw cycles (up to five) is appropriate for cell culture use. Although presented data show no significant reduction in the performance of serum that has been frozen and thawed up to five times we recommend that the number of freeze/thaw cycles serum products are subjected to is minimized.

Recommended storage and handling

We recommend serum to be stored at -10°C or lower. Labeled expiration dates are based on stability studies performed on serum stored at -10°C. This temperature was chosen as it is common for laboratory freezers to be maintained at this temperature. However, studies involving serum stored at -80°C indicate no difference in performance. Once thawed, serum can be stored at 2°C to 8°C for up to six weeks with maintain quality. If serum needs to be stored longer than six weeks after thawing, it is recommended that the serum be aliquoted into convenient volumes and refrozen.

Thawing

Remove serum from frozen storage and place in a refrigerator overnight at 2°C to 8°C. Transfer serum to a 37°C water bath, agitating periodically to mix the solutes concentrated at the bottom of the container. Do not hold the serum at 37°C any longer than necessary after thawing. Thawing serum in a bath above 40°C without mixing might "cook" the concentrated proteins in the bottom of the container and precipitates can form in the bottle. Thawing serum at higher temperatures is not recommended. Alternatively, bottles may be placed directly from frozen storage into a 37°C water bath. Bottles should be agitated to enhance mixing and thawing. Turbidity and flocculent material can be present after thawing or after prolonged storage. Experience indicates that regardless of the method used to thaw serum, it is critical that it be mixed during the thawing process to prevent the formation of gradients and subsequent precipitation. Due to differences in thawing rates of different components, serum will form a gradient if it is not mixed as it thaws. If serum is allowed to remain in such a gradient state, precipitation is likely to occur.

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29193628 AA 02/2016