EquaFETAL® Used in Cell Culture Applications

Cell culture is needed for many applications such as biological therapies, antibody production, regenerative medicines and academic research. In cell culture, researchers need to have a base medium that is supplemented with growth factors to promote cell proliferation. These growth factors primarily come from fetal bovine serum (FBS) which has been shown to be effective for numerous cell lines. The versatility and robustness makes fetal bovine serum the primary supplement source used in cell culture.

The economics of U.S. origin fetal bovine serum are highly volatile due to the difficultly of acquiring and manufacturing material and the demand for current biological applications. The price of fetal bovine serum has increased so much so that researchers have looked for alternatives. Serum-free supplements were the first alternative used by researchers, but they are complex. Previous models show that development of serum free media is not an easy task and developing a supplement may be dependent on the cell type and culture system used.[1] The other alternative is EquaFETAL® (EF) which is 100% bovine serum, but collected after birth of the animal. It is the bio-equivalent solution for stable pricing, lot to lot consistency (Figure 2) and exceptional raw material traceability.[2] Studies have shown that this serum product can be as effective as fetal bovine serum in many cell applications (Figure 1).

Although cost reduction is important in all aspects of business, quality is never compromised. A point of contention when choosing an alternative serum, is to make sure your supplier is reliable. A transparent supplier can provide traceability documentation, certificates of analysis, certificates of origin and cooperate with on-site audits. This is a key factor in risk reduction.[3] EquaFETAL® is always tested for key biochemical composition and reported on certificates of analysis. The similarities in composition to fetal bovine serum and differences to newborn calf serum (NBCS), most notably GGT and IgG, highlight the effectiveness of EquaFETAL® to be used in cell culture applications (Table 1).

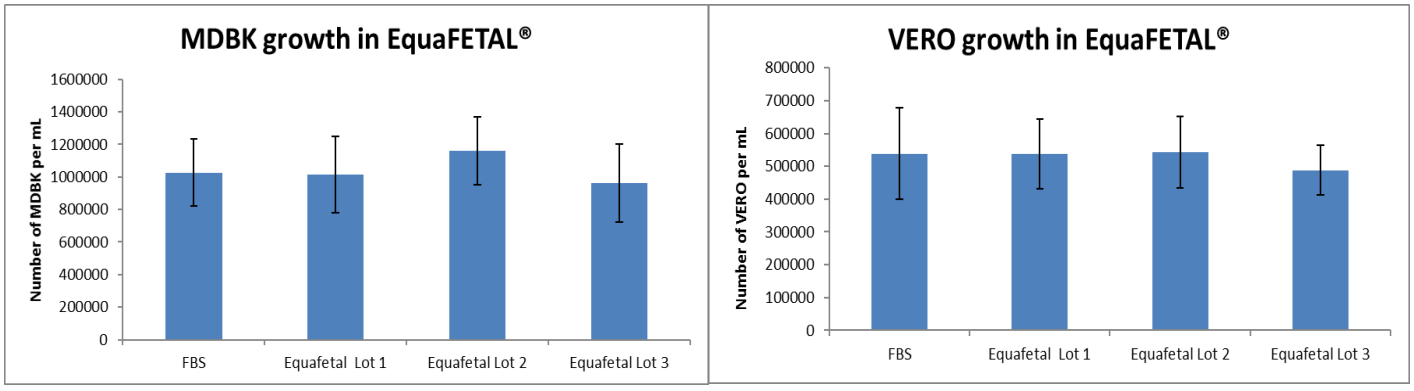

Figure 1: MDBK (Left) and Vero (Right) cell proliferation results in three different lots of EquaFETAL®. MDBK cells were cultured in the presence of 10% fetal bovine serum and 10% EquaFETAL® in DMEM with L-Glutamine for three passages. Cells were seeded between $4x10^4$ to $6x10^4$ cells/cm^2 for each passage. Cell counts were determined using Invitrogen™ Countess™ automated cell counter. Cumulative average number of cells/mL shown for FBS and EquaFETAL® for passages 1-3. Variation among column means is not significantly greater than expected by chance. Standard error between duplicates shown.

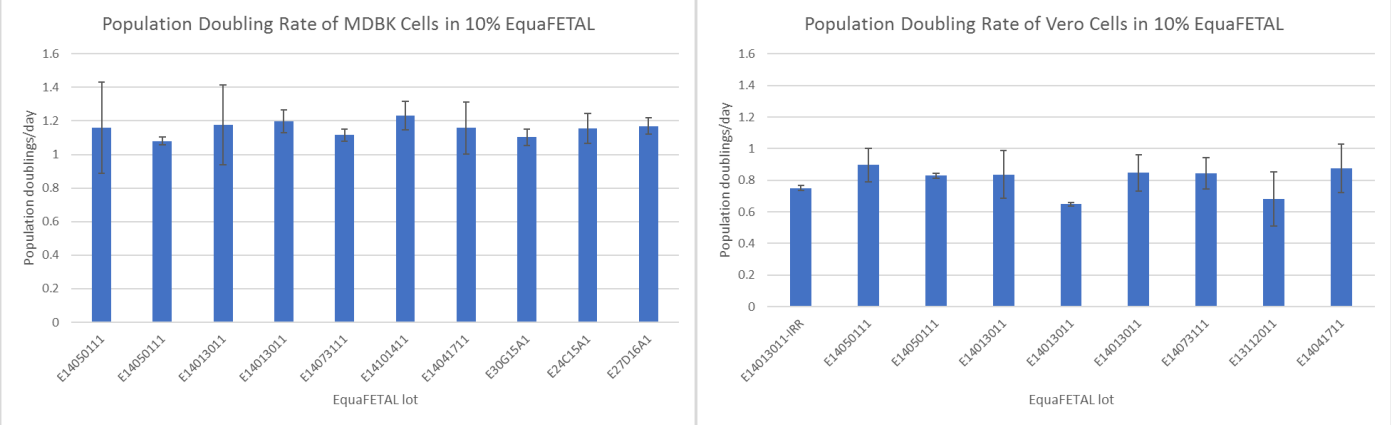

Figure 2: EquaFETAL® bovine serum performs similarly between manufactured lots. MDBK (Left) and Vero (Right) cells were cultured in 10% EquaFETAL® serum in DMEM with L-Glutamine, Penicillin and Streptomycin. Tissue culture plates were inoculated between 4000 and 7000 cells per square centimeter and cultured between 2-4 days for 3 consecutive passages. Suspensions were made by dissociating cells from tissue plates with 0.25% Trypsin-EDTA solution. Cell densities were determined by using the Invitrogen™ Countess automated cell counter on cell suspensions. The counted cells reported were viable as determined by trypan blue exclusion. Multiple lots listed because results were determined from different experiments. IRR = Gamma Irradiated to 30-45 kGy. Error bars shown are standard error between passages (n=3).

Biochemical Component	Newborn n=13	EquaFETAL® n= 11	FBS n= 12	Δ NBCS-EF	Δ FBS-EF	Units
Calcium	11.8	11.7	14.0	0.1	2.2	mg/dL
Chloride	101.6	99.2	94.5	2.4	-4.7	mEQ/L
Iron	83.1	103.3	156.5	-20.2	53.3	µg/dL
Magnesium	3.5	2.6	2.9	0.9	0.3	mg/dL
Phosphorus	9.8	7.6	9.7	2.2	2.1	mg/dL
Potassium	9.7	6.5	11.8	3.2	5.3	mEQ/L
Sodium	157.5	143.1	134.1	14.4	-9.0	mEQ/L

ALP	ND	120.5	261.0	ND	140.5	IU/L
AST	81.7	46.6	23.4	35.1	-23.2	IU/L
Bilirubin	0.6	0.6	0.1	0.0	-0.5	mg/dL
CK	404.3	427.0	149.3	-22.7	-277.8	IU/L
Creatinine	1.3	1.6	2.8	-0.3	1.2	mg/dL
GGT	645.1	15.0	3.6	630.1	-11.4	IU/L

Biochemical Component	Newborn n=13	EquaFETAL® n= 11	FBS n= 12	Δ NBCS-EF	Δ FBS-EF	Units
Bicarbonate	17.5	19.0	12.2	-1.5	-6.7	mEQ/L
Cholesterol	40.5	26.5	28.2	14.0	1.7	mg/dL
Glucose	273.4	155.0	111.5	118.4	-43.5	mg/dL
Triglyceride	54.2	26.5	66.1	27.7	39.6	mg/dL

Albumin	2.7	2.7	2.4	0.0	-0.3	g/dL
Globulin	2.7	1.3	1.2	1.4	-0.1	g/dL
Total Protein	5.7	4.0	3.3	1.7	-0.7	g/dL

Biochemical Component	Newborn n=14	EquaFETAL® n= 15	FBS n= 10	Δ NBCS-EF	Δ FBS-EF	Units
IgG	13150	206.4	132.1	12943.6	-74.3	µg/mL

*ND= No data

Table 1: Biochemical comparison of EquaFETAL® and Fetal Bovine Serum. Analysis was performed by Colorado State University Veterinary Diagnostic Laboratory using a Roche Cobas c501 analyzer. Albumin fractions and percentages determined by gel electrophoresis. 13 lots of Newborn Calf serum, 11 lots of EquaFETAL® and 12 lots of Fetal Bovine Serum were analyzed and averages are reported in table. Immunoglobulin G analysis was determined by ELISA. ELISA was performed on 13 lots of Newborn calf serum, 15 lots of EQUAFETAL® and 10 lots of FBS by Zeptometrix Corp.

1. van der Valk, J., Brunner, D., De Smet, K., Fex Svenningsen, Honegger, P., Knudsen, L. E., Gstraunthaler, G. (2010). Optimization of chemically defined cell culture media - Replacing fetal bovine serum in mammalian in vitro methods. Toxicology in Vitro, 24(4), 1053–1063. https://doi.org/10.1016/j.tiv.2010.03.016
2. https://atlasbio.com/ab-product/equafetal/
3. Siegel, W. (2016). Fetal Bovine Serum: Risk Management. *Bioprocessing Journal*, *15*(1), 22–25.