Letter

# Human AB serum as an alternative to fetal bovine serum for endothelial and cancer cell culture*

*In vitro* methods are widely used in biomedical research and industry, often reducing or replacing animal experiments. However, cell cultures are generally supplemented with fetal bovine serum (FBS), also known as fetal calf serum (FCS), to optimize cell growth. Concerns have been raised regarding both the method used to collect the serum from the donor fetus and the possibility of viral contamination of serum (van der Valk et al., 2004, 2010; Gstraunthaler, 2003; Toldbod et al., 2003; Warncke et al., 2006; Brunner et al., 2010). Thus, the replacement of FBS has become a desirable objective in the field of cell and tissue culture.

Current approaches include the replacement of FBS by human platelet lysates, bovine milk growth factors, human umbilical cord blood, serum and plant extracts, among others (van der Valk et al., 2004; Bruserud et al., 2005; Clavreul et al., 2009; Kocaoemer et al., 2007; Rauch et al., 2011; Tekkatte et al., 2011; Pazos et al., 2004; Gstraunthaler, 2003). Some of these approaches require special formulations to be developed for each cell type.

A promising alternative for culture of human cells is the substitution of FBS with converted human AB serum (HABS), which is routinely tested for viral contamination. HABS supports the propagation of human osteoblasts, chondrocytes, and bone marrow cells (Munirah et al., 2008; Hankey et al., 2001; Yamamoto et al., 2003; Yamaguchi et al., 2002), as well as glioma and melanoma cancer cell lines (Clavreul et al., 2009; Pandolfino et al., 2010). These studies suggest that HABS may be a good candidate to replace FBS as a supplement for cell culture.

We tested this using colorectal (HT-29), cervical (HeLa-229), and breast (MCF 7) cancer cell lines routinely used in cancer research, as well as primary human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HuDMEC).

HT-29 and HeLa 229 were from the European Collection of Cell Culture (Salisbury, UK); MCF 7 was a gift from the Institute for Cancer Studies (School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, UK). Media were from Gibco (UK). Cancer cell lines were maintained in McCoys (HT-29), DMEM with sodium pyruvate (HeLa), and DMEM without sodium pyruvate (MCF 7) medium supplemented either with 50 ml of human AB converted serum (HABS; PAA Laboratories, UK) or FBS (Invitrogen, UK).

HuDMEC cells, a gift from Dr J. L. Burn (Department of Oncology, School of Medicine and Biomedical Sciences, Sheffield, UK), were grown in EBM-2 medium (Cambrex Bio Science Inc, UK) supplemented with EGM-2 MV SingleQuots Supplements and Growth Factors kit (Cambrex Bio Science Inc, UK). HUVEC were prepared from healthy umbilical cords (Sheffield Research Ethics Committee (STH 13759) by collagenase digestion (2 mg/ml, Sigma-Alrich, UK). 5% HABS-supplemented cells were grown in human placental collagen type IV (5 $\mu$g per cm2 dissolved in 0.25% acetic acid) (Sigma-Aldrich, UK) pre-coated T-75 flasks (Nunc, UK). 5% FBS-supplemented cultures were grown in 0.2% bovine gelatine (Sigma-Aldrich, UK) pre-coated T-75 flasks. Flasks were washed twice with PBS (Invitrogen, UK) after coating.

Cells were diluted to 2 x $10^3$ cells per 200 $\mu$l full growth medium per well and placed in quadruplicates on uncoated, collagen IV (primary cells, HABS group) or gelatin pre-coated (primary cells, FBS group) 96-well plates. Medium was changed after 4 h and cells were incubated at 37°C in 5% $CO_2$. Proliferation was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay (Sieuwerts et al., 1995) after 1, 2, 3, 4, and 5 days. Absorbance was measured at 540 nm using a MRX II microtiter plate reader using revelation software (Dynex, UK). The means were calculated for each quadruplicate, and the overall mean and standard deviation (SD) were calculated for each data point. Calibration curves were constructed by plating increasing densities of cells in triplicates and measuring their MTT metabolism,

* This work was funded by The Lord Dowding Fund, UK.

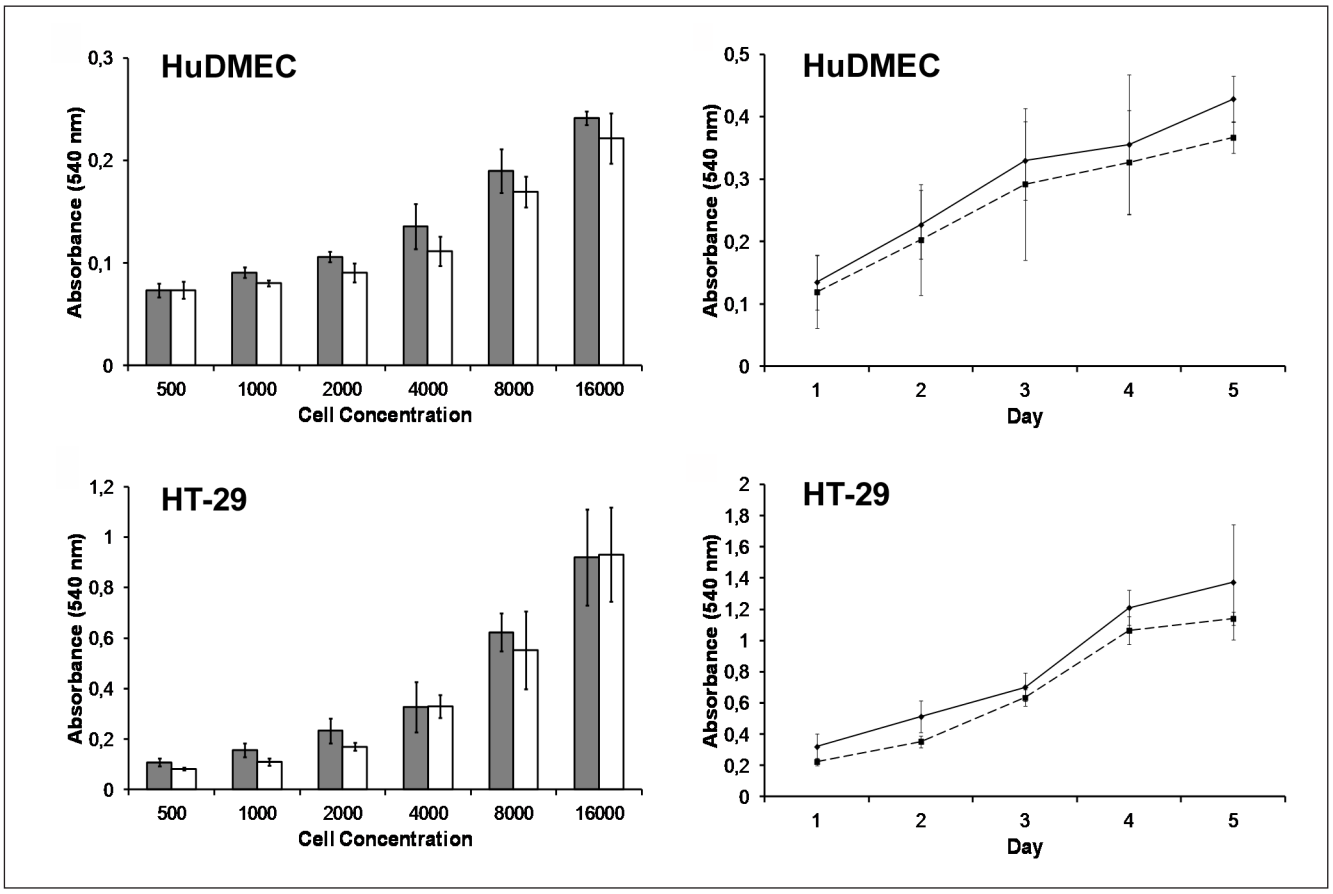


**Fig. 1: Relation between cell number and formazan production and comparison of growth with FBS and HABS shown exemplarily for HuDMEC and HT-29 cells**

Left panel: Increasing cell concentrations were plated (in triplicate) and cultured either with FBS- (solid bar) or HABS- (open bar) supplemented medium. Formazan production was measured after 24 h. Each data point represents the average of three (n=3) separate experiments ±SD. All n.s., Mann-Whitney U-test. Right panel: 2,000 cells/well were plated (in quadruplicate) and formazan production was measured on days 1, 2, 3, 4 and 5. Each data point represents the average of three (n=3) separate experiments ±SD. All n.s., Mann-Whitney U-test. Comparable observations were obtained for HUVEC, HeLa 229, and MCF 7 cultures (not shown).

as above, on day one. Mean absorbance values were calculated for each triplicate, and the overall mean and standard deviation (SD) were calculated for each data point.

We found that HABS supported the growth of all primary cultures of endothelial cells and cancer cell lines tested. Increasing numbers of cells correlated with increasing absorbance levels in all cells studied (shown exemplarily in Fig. 1, left panel). Over a 5-day period, numbers of endothelial cells and cancer cells increased in parallel in HABS-supplemented and FBS-supplemented cultures, as evidenced by increasing formazan metabolism (shown exemplarily in Fig. 1, right panel). No statistically significant differences were observed between HABS- and FBS-supplementation over this time period for any of the cell types.

A drawback of using HABS for cell culture is the cost. Commercial human serum can be up to 4-5 times more expensive than FBS. Here, we used serum concentrations of 10% and 5% for cancer and endothelial cells. However, it may be worthwhile to explore whether lower human serum levels in media would be able to support cell cultures without compromising cell growth.

In conclusion, human serum supported the growth of primary endothelial cells and cancer cell line cultures, and it seems to be a good candidate to replace FBS as a medium supplement in cell culture.

## References

Brunner, D., Frank, J., Appl, H., et al. (2010). Serum-free cell culture: the serum-free media interactive online database. *ALTEX 27*, 53-62.

Bruserud, O., Tronstad, K. J., and Berge, R. (2005). In vitro culture of human osteosarcoma cell lines: a comparison of functional characteristics for cell lines cultured in medium without and with fetal calf serum. *J Cancer Res Clin Oncol 131*, 377-384.

Clavreul, A., Jean, I., Preisser, L., et al. (2009). Human glioma cell culture: two FCS-free media could be recommended for clinical use in immunotherapy. *In Vitro Cell Dev Biol Anim 45*, 500-511.

Gstraunthaler, G. (2003). Alternatives to the use of fetal bovine serum: serum-free cell culture. *ALTEX 20*, 275-281.

Hankey, D. P., McCabe, R. E., Doherty, M. J., et al. (2001). Enhancement of human osteoblast proliferation and phenotypic expression when cultured in human serum. *Acta Orthop Scand 72*, 395-403.

Kocaoemer, A., Kern, S., Kluter, H., et al. (2007). Human AB serum and thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for the expansion of mesenchymal stem cells from adipose tissue. *Stem Cells 25*, 1270-1278.

Munirah, S., Ruszymah, B. H., Samsudin, O. C., et al. (2008). Autologous versus pooled human serum for articular chondrocyte growth. *J Orthop Surg 16*, 220-229.

Pandolfino, M. C., Saiagh, S., Knol, A. C., et al. (2010). Comparison of three culture media for the establishment of melanoma cell lines. *Cytotechnology 62*, 403-412.

Pazos, P., Boveri, M., Gennari, A., et al. (2004). Culturing cells without serum: lessons learned using molecules of plant origin. *ALTEX 21*, 67-72.

Rauch, C., Feifel, E., Amann, E. M., et al. (2011). Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. *ALTEX 28*, 305-316.

Sieuwerts, A. M., Klijn, J. G., Peters, H. A., et al. (1995). The MTT tetrazolium salt assay scrutinized: how to use this assay reliably to measure metabolic activity of cell cultures in vitro for the assessment of growth characteristics, IC50-values and cell survival. *Eur J Clin Chem Clin Biochem 33*, 813-823

Tekkatte, C., Gunasingh, G. P., Cherian, K. M., et al. (2011). "Humanized" stem cell culture techniques: the animal serum controversy. *Stem Cells Int*, 504723.

Toldbod, H. E., Agger, R., Bolund, L., et al. (2003). Potent influence of bovine serum proteins in experimental dendritic cell-based vaccination protocols. *Scand J Immunol 58*, 43-50.

van der Valk, J., Mellor, D., Brands, R., et al. (2004). The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. *Toxicol In Vitro 18*, 1-12.

van der Valk, J., Brunner, D., De Smet, K., et al. (2010). Optimization of chemically defined cell culture media--replacing fetal bovine serum in mammalian in vitro methods. *Toxicol In Vitro 24*, 1053-1063.

Warncke, M., Dodero, A., Dierbach, H., et al. (2006). Murine dendritic cells generated under serum-free conditions have a mature phenotype and efficiently induce primary immune responses. *J Immunol Methods 310*, 1-11.

Yamaguchi, M., Hirayama, F., Wakamoto, S., et al. (2002). Bone marrow stromal cells prepared using AB serum and bFGF for hematopoietic stem cells expansion. *Transfusion 42*, 921-927.

Yamamoto, N., Isobe, M., Negishi, A., et al. (2003). Effects of autologous serum on osteoblastic differentiation in human bone marrow cells. *J Med Dent Sci 50*, 63-69.

David Cánovas and
Nigel Bird*

Liver Research Group,
Oncology Department, School
of Medicine and Biomedical
Sciences, University of
Sheffield, Sheffield, UK

*Corresponding author:
Nigel Bird, PhD
E-Floor, Oncology Department
School of Medicine
and Biomedical Sciences
University of Sheffield
Beech Hill Road
Sheffield S10 2RX
UK
Phone: +44 11422268018
e-mail: N.Bird@sheffield.ac.uk