Comment

A Severe Case of Fraudulent Blending of Fetal Bovine Serum Strengthens the Case for Serum-free Cell and Tissue Culture Applications

In 2011, GE Healthcare (a unit of General Electric Co.) acquired PAA Laboratories, Linz, Austria. In April 2013, GE Healthcare published a product information to customers, stating that batches of fetal bovine serum (FBS) produced at PAA facilities from March 2008 to March 2013 are subject to label non-conformances, i.e. that:

"These products may contain added adult bovine serum albumin (BSA) of United States origin, water, and/or cell growth promoting additives. For FBS product shipped into countries other than the United States, current product labeling states that the origin of the product is either Australia or EU approved serum sources. In addition to, or instead of product of this origin, the product may contain adult BSA of United States origin and/or may contain FBS from sources including United States, Canada, Argentina, Brazil, and/or Mexico."

This warning of GE Healthcare about the purity and quality of FBS from PAA Laboratories prompted us to write a note to inform and to alert the cell culture community, and to provide background information about FBS, and about serum alternatives and serum-free cell culture applications, respectively.[1]

FBS is a natural cocktail of most of the factors required for cell attachment, growth and proliferation, effective for most types of human and animal (including insect) cells.[2] Although in use as a universal growth supplement of cell and tissue culture media for more than 50 years, FBS has never been fully characterised. Recent proteomic and metabolomic studies revealed approximately 1,800 different proteins[3,4] and more than 4,000 metabolites present in serum,[5] with the proportions of each of these components varying between different serum batches. Furthermore, global supply and availability of FBS has changed dramatically over the past few years. FBS is a by-product of the beef packing industry. Thus, FBS supply is dictated by many factors, including beef consumption (e.g. more white meat over red meat), feed prices, environmental factors such as drought, cattle import and export, governmental farm policies,[6] and the outbreak of diseases (e.g. foot and mouth disease, BSE).[7–10]

From this, it can be concluded that the use of serum in cell culture may involve a number of disadvantages: a) serum in general is an ill-defined supplement in culture media, with high qualitative and quantitative, geographical and seasonal batch-to-batch variations; b) FBS may contain adverse factors, like endotoxins, mycoplasma, viral contaminants or prion proteins; c) there are animal welfare concerns surrounding the harvest and collection of FBS from unborn bovine fetuses; and d) FBS availability is dependent on the global market.[11–13]

There is a severe geographical mismatch between the supply of, and the demand for, FBS. Demand is highest in the USA and Europe, while the major sources of FBS are far away from these areas — in Brazil, Argentina, South Africa, Australia, New Zealand, and Central America. It is in these countries that huge meat cattle herds — bulls and cows — roam freely together, and as a result, many cows are pregnant at the time of slaughter.[13,14] The same holds for the geographical distances between raw serum producers and FBS processors. The latter are also mainly located in the USA and in Europe. It is estimated that approximately 500,000 litres of FBS are sold per year, which means that more than 1,000,000 unborn bovine fetuses have to be subjected to the harvesting procedure — a fact that raises major animal welfare concerns,[13–16] and indeed the numbers are still increasing. As a consequence, a number of strategies were developed in terms of the Three Rs,[17] to reduce or replace the requirement for FBS in cell culture media.[15]

As well as concerns about the number of animals required to supply the FBS market, there are additional concerns that this market is only loosely regulated[18–21] — and this creates opportunities for abuse.[18,19,22] This abuse has been evident in the past, and is most likely still happening now. For example, in 1994 it was reported[20] that approximately 30,000 litres of "New Zealand" FBS was sold worldwide. However, only 15,000 litres of high-quality FBS were annually collected in New Zealand. Even now, exact figures for the global FBS production rate are still unavailable, which raises suspicions as to whether FBS in general might be blended with other sera to meet increasing demands. No attempts have ever been

undertaken to trace the collected sera, in order to gain clear evidence about their geographical origin.

Obviously, in the last 20 years nothing has changed.[18–20] As pointed out above, many FBS batches were blended with bovine serum albumin, water and growth promoting additives.[1] The US Food and Drug Administration (FDA) reports that 143 batches of FBS, amounting to a total of approximately 280,000 litres, are affected.[23] This latest incident might be just the tip of the iceberg. *Most importantly*, the actual case might also have a substantial impact on many thousands of cell and tissue culture experiments, and, in particular, where GLP and GMP conditions are required, this can hardly be ignored!

This recent fraudulent action should be taken as an opportunity to question the use of FBS as cell culture media supplement. We therefore appeal to cell and tissue culturists to reduce or completely avoid FBS in their cultures, and to turn to other options, e.g. serum-free cell and tissue culture,[15,16,24] or the replacement of FBS by the use of serum substitutes, such as human platelet lysates.[25–27] In particular, cultures that are newly initiated should be grown from the very beginning under serum-free conditions. In 2003 and 2009, European cell culture experts gathered at two workshops to discuss options for, and the methodologies of, serum-free cell culture. Two comprehensive workshop reports were published,[15,16] in which clear recommendations for the replacement of FBS, and for the design of serum-free media, respectively, are provided. Following these report recommendations will result in:

— scientifically better (and more-reproducible) data;

— safer products;

— ethical research without harming animals;

— better availability of cell and tissue culture media;

— the transparent and traceable composition of culture media; and

— a significant contribution to Good Cell Culture Practice[28] (GCCP).

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