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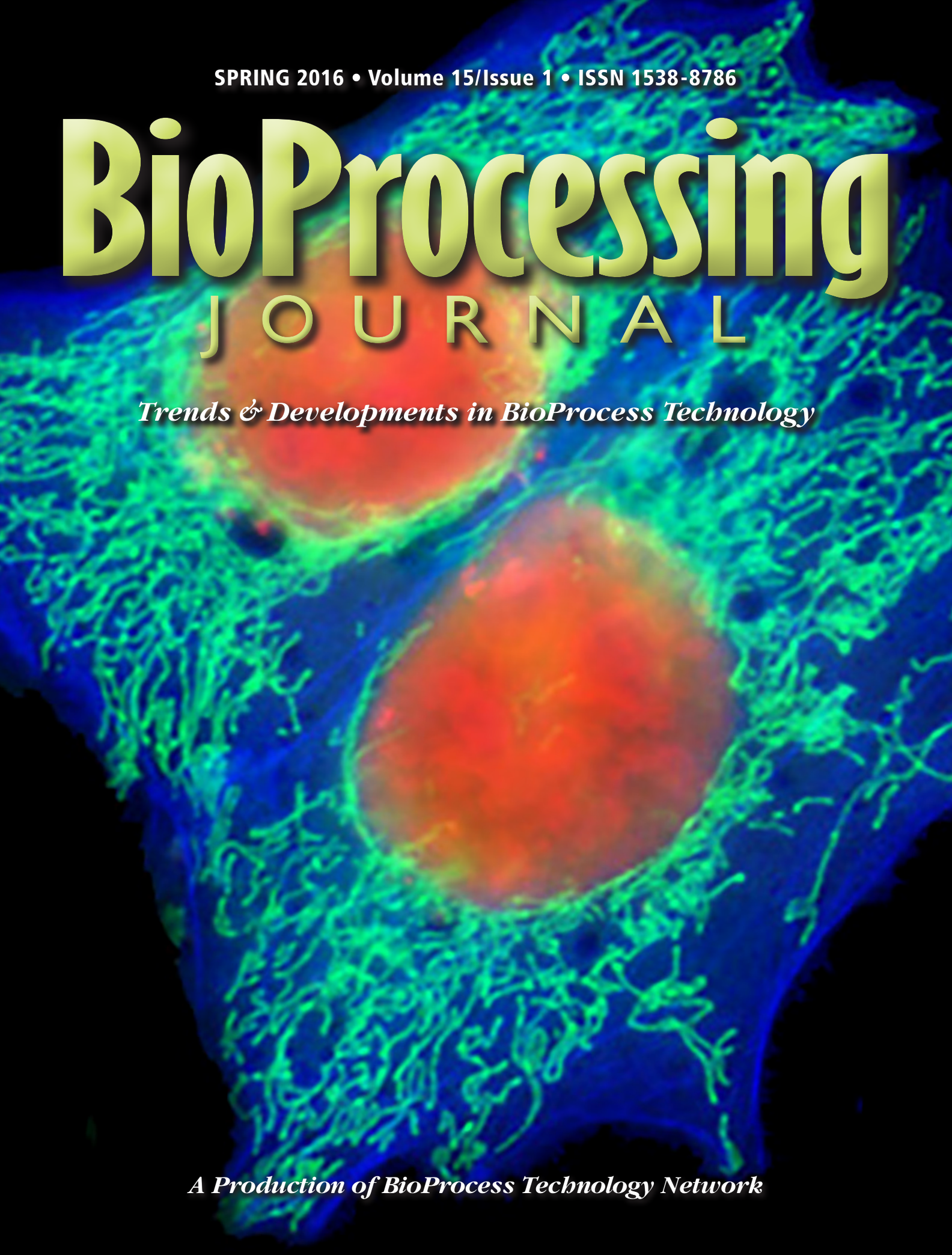
Trends & Developments in BioProcess Technology



A Production of BioProcess Technology Network

BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

ISBIOTECH CONFERENCE EXCLUSIVE

Serum: *What, When, and Where?*

By Rosemary Versteegen

Abstract

For over 80 years, fetal bovine serum (FBS) and other animal-derived materials have been widely used in the production of vaccines, and more recently, biotherapeutics, for both human and animal applications. Ever since FBS was initially developed as a cell culture reagent, there have been efforts made to avoid the use of this critical commodity. The International Serum Industry Association (ISIA) recognizes the requirement for robust risk assessment and management, and has several ongoing programs designed to help mitigate the risk of using animal-derived materials. This article will provide an outline of the state of the industry and of these programs.

Introduction

As far back as the 1940s, the use of animal sera in mammalian cell culture has been key in the development of biotechnology. The injectable polio vaccine, developed by Jonas Salk in the early 1950s, was one of the first reagents mass-produced using cell culture techniques, and played a significant role in reducing infection rates amongst children. Animal-derived components continue to be critical in the production of many healthcare-related products and have never been directly linked to adverse impacts in humans.

Current cell therapy techniques require the use of human serum to maintain autologous transplant tissue while being manipulated. Regulators worldwide recognize that there is an inherent risk in the use of a human-derived material to support human cells prior to transplant. It should be noted that the potential risk of contamination from a human-to-human model far outweighs the risk of transmission of viral contamination from an animal-derived material.

It has been asked why, if meat from many countries can be imported and eaten, serum from these same counties is of concern when used in biotechnology manufacturing. The possibility of enrichment of adventitious agents during cell culture presents a higher level of possible risk and must be managed accordingly. As a result, most regulatory bodies only allow the use of serum and animal-derived materials when their use can be justified because there is no viable alternative.[1] The potential for adventitious agent contamination is a recognized risk in using animal-derived materials. Although contamination can occur at many points in manufacturing, it is frequently difficult to determine the root-cause with certainty. What is certain is the cost in time, production, and clean-up expense of a facility contamination, whatever the cause. Advances in testing and filtration technology in recent years have helped in the investigation and management of such risk.

As a result of these concerns, many attempts have been made to replace these animal-derived materials in mammalian cell culture. In the late 1980s and early 1990s, a search began for the "Holy Grail" of cell culture intended to replace serum. It would cost thousands of research hours, millions of dollars, and ultimately prove to be unsuccessful. Since that time, it has become apparent that animal-free or chemically defined media, or serum replacements, can be developed for certain cell lines and applications—if enough time and money is spent—although these efforts may fall short in terms of the optimal yield of the desired product. It should also be recognized that animal material-free replacements are not without their own concerns. For example, plant-derived materials implicate both contaminating animal and less understood plant-derived adventitious agents in downstream processes.

It seems clear then that although the use of serum has been "going away" for more than 50 years, there continue to be applications where there is no viable alternative. Fetal bovine serum (FBS), in particular, has not been outperformed as a general reagent in providing the optimum breadth and intensity of cell growth stimulation. It is therefore important to understand and fully utilize all available methods to manage and reduce risk. At this time, the focus of this risk management effort must be on sourcing, traceability, and treatment.

Sourcing

State of the Industry: The serum world globally has recently undergone a series of changes both in the supplier and industry arenas. The numerous mergers and subsequent divestitures of large serum businesses have impacted historical sourcing arrangements[2] which has weakened previously strong sourcing relationships. In recent years, cattle herds have been depleted due to sustained drought

conditions and other agricultural issues, and are now being rebuilt currently in the USA.[3] In some geographies, the availability of FBS, a by-product of the meat industry, is in flux due to fewer in-calf cows coming to slaughter. It should be noted that, on a global basis, a shortage in one geography is often balanced by increased availability in another.

The three main aspects to be contemplated when considering the source of serum, particularly for biotechnology applications, are: (1) country disease profile; (2) country robustness of infrastructure; and (3) the supplier.

Country Disease Profile

As discussed by Siegel and Foster[4], the most desirable geographic areas of the world are those where diseases and adventitious agents are not found in the cattle populations. As a country that has never had a case of bovine spongiform encephalitis ("mad cow") or foot-and-mouth disease, New Zealand ranks highest in this category. Unfortunately, as a relatively small country, New Zealand can only account for around 6% of the world's FBS supply. The recent reclassification of the USA to "negligible risk" by the World Organization for Animal Health (OIE)[5] has established US-origin FBS as having a similar risk profile to that from New Zealand and Australia.

Country Robustness of Infrastructure

The second aspect based on geography is the credibility and robustness of the regulatory environment within the country of origin. The consistency, veracity, and intensity of inspection and regulatory oversight are perhaps even more significant than the written regulations, in terms of building credibility in this arena. Concerns around risks to animal and human health are codified worldwide. The significant costs of clean-up and lost trade resulting from animal disease outbreaks have further heightened awareness. Examples include the foot-and-mouth disease outbreaks in the UK (2001 and 2007) and Japan (2010), and the porcine epidemic diarrhea virus (PEDv) and highly pathogenic avian influenza virus (HPAIv) outbreaks in the USA (2013 and 2015). These events have led to ever-stronger regulations surrounding animal management and the import/export of animal by-products.

The Supplier

In recent years, tighter EU regulations regarding the import of animal-derived materials have been enacted, and countries successfully supplying these products have adapted to the more stringent regulations and specific standards. This has had an impact on various geographies worldwide by bringing about a move toward standardizing animal health status and veterinary documentation. ISIA considers standardization and harmonization to be important and has, for example, worked with the US Pharmacopeia (USP) on Chapters 1024 and 90 to standardize QC testing and help minimize the confusion for the customer in comparing test results.

The need for reliable quality and quantity is an important aspect in the decision process of selecting a supplier. A good supplier is a transparent supplier, and one who becomes a trusted supply chain partner. Trust is an important component of the serum supplier relationship, one that ISIA strives to significantly enhance through its cornerstone Traceability Certification Program.

Traceability

At the first meeting of the newly formed ISIA in July 2006, the necessity for a program to support traceability was agreed upon. Association members recognized the need for self-policing based on the fact that

The International Serum Industry Association (ISIA) was founded in 2006 to represent collectors, producers, sellers, distributors, and end-users of animal sera and other animal-derived materials worldwide. At this time, members of the association provide greater than 90% of the animal sera and animal-derived products used in life science research, and biotherapeutic, vaccine, and *in vitro* diagnostic manufacturing.

Fifty-four (54) companies are currently full or associate members of the association, and 67% of those who have been members for more than two years and are eligible for ISIA Traceability Certification have achieved that status or are actively in the process of working towards it. The Traceability Certification Program is an ISIA cornerstone and will be described in more detail later in this document.

In the decade of its existence, ISIA has become a key player in helping industry, end-user, and regulatory decision-makers understand the complexity of these markets and products, and is now viewed by many as a valued partner.

Further details may be found on the ISIA website:

www.serumindustry.org

regulators and customers had a collective low regard for the industry, which showed no signs of improving at that time. This focus is clearly enunciated in the Mission of the Association.

> **ETHICS** — **ISIA** shall establish, promote, and assure compliance with uncompromised standards of excellence and ethics in the business practices of the global animal serum and animal-derived products supply industry.
>
> **SAFETY AND SAFE USE** — Our primary focus will be on safety and safe use of serum and animal-derived products through proper origin traceability, truth in labeling, and appropriate standardization and oversight.
>
> **EDUCATION** — We will work to educate stakeholders on the scientific foundation of the safe use of serum and animal-derived products.

A mass-recall incident of adulterated and misbranded FBS in 2013 impacted a large number of suppliers and customers and was deeply shocking to customers and suppliers alike. Although this incident had the potential to reinforce negative views of the serum industry, it also presented an ideal opportunity to showcase the ISIA Traceability Certification Program. After analysis of this incident, it was determined that an ISIA traceability audit would have revealed the fraud that had escaped detection by many standard audits. This episode demonstrates the reason for the increasing demand by customers and regulators for ISIA traceability certification.

So what is the ISIA Traceability Certification Program? Since the first pilot run of the ISIA Traceability Certification audit in late 2009, the program has evolved considerably and continues to be upgraded to meet industry needs. The policy and audit checklist are designed to ensure that an accurate paper trail is maintained at every step of the manufacturing process, from the abattoir to the end-user. The audit must be performed by a third party auditor approved by ISIA following an ISIA-approved audit plan. The audit is modeled after the International Organization for Standardization (ISO) and can be combined with an ISO audit.

At every hand-off, a mass-balance is performed: how much came in at what cost, and how much moved on to the next step at what cost. The audit is designed in modules so companies can be certified for those aspects of sourcing, manufacturing, or distribution that are relevant. This way, companies can be certified for the full range of activities or just those aspects that they perform.

In addition, the scope of the audit covers all serum in a facility, no matter what type or source, or designated customer. This broad overview of all records provides a broader basis for certainty that the documentation being reviewed is accurate. ISIA continues to work to strengthen the program, and has already expanded the scope to include bovine serum albumin (BSA). Current efforts include a study of various ways to identify the geography of origin.

Geographic Identification

The study of stable isotopes, and their role in identifying the geography of origin of food, has been evolving over the last 25 years. Studies on the ratios of oxygen and hydrogen from water, and carbon and nitrogen in fast food[6] indicated that it might be possible to identify food origin locations in the USA based on these isotopic geotags. With that in mind, ISIA began to investigate the potential for identifying the origins of serum products. Early results using material from clearly identified sources were promising, so further investigation was undertaken (**Figures 1** and **2**). The study

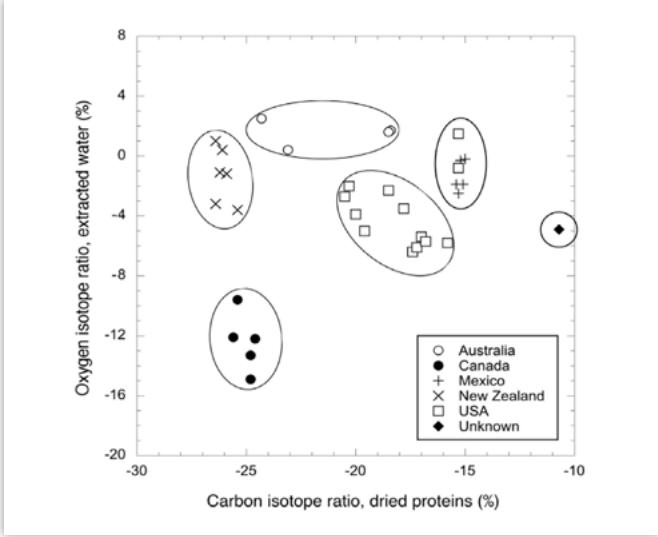


FIGURE 1. Pilot Study 1—by country.

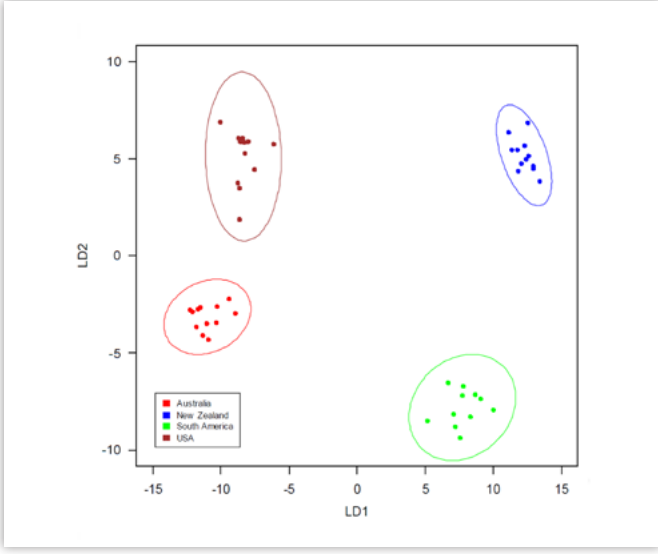


FIGURE 2. Pilot Study 2.

of trace elements from soil as markers of geography has been a more recent development, but has also been shown to demonstrate the source of a variety of materials. A pilot study undertaken using serum from various corners of the world again showed the potential for geographic identification. Independently, statistical analysis of US Geological Survey (USGS) data available on the internet showed that soil analysis from cattle-raising areas of the USA could be distinguished.[7]

Encouraged by these results, ISIA is in the process of designing a larger study to validate the use of such methods for serum source identification. This is seen as a potentially valuable addition to the Traceability Certification Program detailed above. An update on the status of this work will be presented at the Annual General Meeting of the Association in Barcelona in May 2016. Details are available on the ISIA website: www.serumindustry.org.

Treatment

Terminal sterilization is not an applicable methodology for biologically active components such as serum. Instead, the user is restricted to post-manufacturing treatment alternatives. High-temperature short-time (HTST) treatment does not work for serum because of variability in biological activity. UV treatment is not economically viable for use with serum. Heat treatment at 56°C for 30 minutes is an older methodology and is neither efficient nor particularly effective.[8] ISIA has and continues to recommend gamma irradiation as the treatment of choice for serum being used in biotherapeutic applications and vaccines.

Gamma Irradiation

This methodology is remarkably efficient in the reduction of viral load for all but very small, non-enveloped viruses such as parvovirus, thus significantly reducing risk.[8] Over the years, it has become apparent that there is a great deal of uncertainty among regulators and users about all aspects of this methodology. ISIA has recently convened a task force comprised of member companies, customers, regulators, and irradiators to produce a white paper delineating all aspects of the process. This will include transportation issues to and from the irradiator, validation of load configuration during irradiation, and effectiveness of irradiation among other related aspects. This will be published as a series of documents, each one providing easily accessible information on this subject.

Conclusion

When ISIA was founded in 2006, regulators in government and industry viewed animal-derived materials, particularly FBS, as a suspect raw material that lacked adequate means of control for quality and sourcing. ISIA continues working to dispel this notion by championing the Traceability Certification Program, which promotes ethical behavior by all involved, as well as providing increased comfort of maximal risk management.

ISIA strives to continuously improve the understanding and credibility of this dynamic industry and serve all of its supporting members, customers, and regulators. Please let us know how we can help you.

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