Serum: A Better Characterized Biological

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Abstract

The International Serum Industry Association (ISIA) was founded in 2006 to represent collectors, producers, suppliers, distributors, and end-users of animal serum and other animal-derived materials worldwide. The current membership of the Association provides greater than 90% of the animal serum and animal-derived products used in life science research, and bio-therapeutic, vaccine and *in vitro* diagnostic manufacturing. The ISIA supports all initiatives to protect animal and human health and the requirement for robust risk assessment. The Association has several programs operating that are designed to help mitigate the risks associated with the use of animal-derived materials. The current state of these programs will be reviewed.

Introduction

Serum and other blood-derived products continue to play a critical role in the biomedical and biopharmaceutical arenas. The continued and increasing use of this material over the last 60 plus years has contributed enormously to the fight against human and animal disease. The use of animal derived products does, however, raise concerns. The potential for the introduction of adventitious agents into cell cultures through animal-derived medium supplements and reagents, and their subsequent replication during cell culture is a frequently discussed topic. As a result, most regulatory bodies allow the use of animal serum and other animal derived materials only when their use can be justified, and where there is no viable alternative.[1,2]

As a result of these concerns surrounding the perceived risks associated with the use of serum in cell culture, many attempts have been made to remove or replace animal-derived materials in mammalian cell culture. Starting in the late 1980s, thousands of research hours and millions of dollars were spent seeking a replacement, with only partial success. It has since become apparent that animal-free media or serum replacements may be developed for some specific cell lines and applications, but not for others. It must also be recognized that

animal material-free replacements are not, themselves, without risk. Plant-derived materials, for example, can introduce both animal and plant derived adventitious agents into cell cultures.[3]

Current applications that may require the use of serum include:

1. Legacy products where serum is specified in the manufacturing protocol
2. Therapies requiring the growth of primary cells
3. Products requiring extensive post-translational modification
4. Areas where speed to market is essential

Approach to Risk Management

Fetal Bovine serum (FBS) has long been found to be the best and most broadly applicable growth supplement. Details of the collection, processing and testing of FBS and other bovine sera have been detailed by the USP,[4,5] among other worldwide agencies. ISIA has undertaken a variety of approaches to the issue of risk management. These are largely applicable to other animal sera and animal derived products.

Traceability

FBS is collected from cattle slaughtered for human consumption and is therefore a by-product of the meat industry. All major meat producing countries around the world are therefore potential sources of FBS. Currently, the highest volumes are collected in Brazil, the USA and Australia.[6] Movement of animal derived material between countries is heavily controlled in the interests of animal health, with significant variations in the import regulations between different geographies.

Serum from all geographies is used widely in life science research. Biopharmaceutical and vaccine production use material sourced from countries offering a low risk of infectious disease and a robust infrastructure for animal management. At this time, the geographies of choice for manufacturers are New Zealand, Australia and the USA. The rational is as follows: from an animal health standpoint, neither New Zealand nor Australia has ever had a case of Bovine Spongiform Encephalitis (BSE), neither do they have endemic Foot and Mouth Disease (FMD).[7] The OIE has recently declared the USA as a negligible risk country for BSE,[8] and FMD is not present in the USA. All three counties are also perceived as having extremely robust and well enforced regulations and control of animals and their derivatives. It should be noted that at this time these three geographies account for around 45% of the FBS collected worldwide

ISIA Traceability Certification

Given the perceived importance of source and the associated financial premium, product traceability was an early objective for the ISIA. The program began in 2009 and has been continuously improved and strengthened over time.

Table 1. ISIA traceability audit modules.

Module	Section
A	Auditor and Company Guidelines
B	Overview of Company Quality Systems
C	Raw Materials
D	Blood Collection
E	Blood Transfer – Collection to Primary Processing
F	Primary Processing – Blood Processing to Serum or Plasma
G	Serum and/or Plasma Transfer – Primary Processing to Final Processing
H	Secondary Processing – Finishing of Serum or Plasma
I	Serum and/or Plasma transfer to other locations (including intercompany)
J	Finished Material
K	Shipping
L	Bovine Serum Albumin Manufacturing
M	Auditor Summary

Traceability certification requires an acceptable audit by an approved, independent, trained auditor from a recognized auditing company, to an audit plan developed jointly by the CEO of the Association, the auditor, and the company seeking certification. This ensures that all aspects of a frequently complicated business can be properly covered. The audit itself is based on a Traceability Policy, which includes penalties for noncompliance, supplemented by a detailed check list. The checklist is modular and covers the steps in the collection, manufacturing and distribution process outlined in Table 1.

It should be noted that the inclusion of Module L, Bovine Serum Albumin Manufacturing, is the first step in the expansion of the program to animal derived materials other than serum.

The policy and checklist allow for tracking of material from the abattoir to the end user. Companies can be audited for those steps in the process that they perform, thus allowing for complete coverage of all steps in the chain. All sera in a facility are subject to audit to ensure the processes are robust. It must be noted that certification cannot be bought, a distributor cannot claim certification if their ordering and shipping capabilities have not been subjected to audit.

Particular attention is paid during the audit to the transfers between departments or companies and a standardized spreadsheet is available to calculate the financial and mass balance at each step. This, together with the fact that all transactions in a company must be open to audit, are the two critical factors that differentiate ISIA traceability from other audits.

This detailed audit of documentation is a first step in ensuring that material purchased is as represented. Traceability certified companies are listed on the ISIA website and may utilize the ISIA Seal on marketing materials (Figure 1) At this time 20 companies are certified worldwide, representing more than 70 percent of the material used in the manufacturing of biotherapeutic molecules, vaccines and diagnostics.

Figure 1. ISIA seal for use by Certified companies.

Geographic Origin

ISIA has been researching ways to identify the geographic origin of materials since 2008. Pilot experiments designed by the Association using stable isotopes, a widely used and accepted technique in the food industry,[10] demonstrated that some level of resolution could be attained. However, the inability to differentiate between Texas and Mexico was seen as a concern.[11]

Further study revealed that trace element analysis was more applicable to serum. It has been well established that soil geochemistry and climate patterns differ by geography. Plants take up chemicals from their environment in a ratio consistent with the local geography and form a "chemical fingerprint". Likewise, ruminant animals, such as cattle, acquire a chemical fingerprint relating to their origin through consuming local food and water (Figure 2).

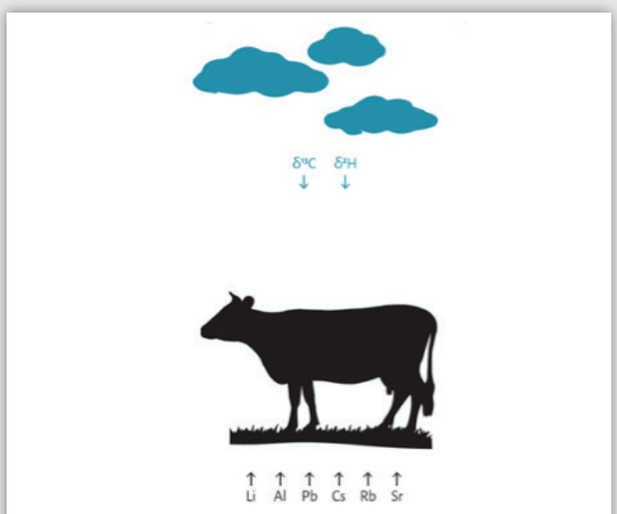

Figure 2. Chemical fingerprint development.

ISIA has established a partnership with Oritain Global Ltd, a New Zealand based company, to build an industry database which can then be used to certify origin of serum. The data base will initially contain the results from more than 1000 serum samples from eight important sourcing geographies, and will continue to be strengthened and amplified as time progresses.

Oritain analyzes serum products through chemical methods and statistical interpretation to determine the chemical fingerprint of origin. A comparison can be made with material acquired at any point in the supply chain to a pre-developed database, ensuring traceability claims are accurate.

To develop the unique fingerprints of sera from different countries, a sample of serum or plasma is first prepared for and analyzed with inductively coupled plasma mass spectrometry (ICP-MS) following established analytical chemistry techniques. Results are then corrected for blanks and data quality evaluated. Statistical analysis is carried out on a subset of analyzed elemental concentrations using Oritain's proprietary statistical algorithm. Statistical data quality was checked for normality and any outlying data-points are identified and validated with analysis repeats. Sample set size is also checked with general methods to validate that the subset size is appropriate. Outputs include univariate and multivariate statistical analyses using a combination of general exploratory and discriminatory models.

Early work, as shown in Figure 3, provide evidence of the ability to differentiate between several important geographies. Each point corresponds to the chemical fingerprint of one serum production lot. Points which have similar chemical fingerprints (and related origin) appear close together in space. Different origins/fingerprint groups appear as separate clusters.

In verifying an origin of serum, a tested sample is compared back to the reference fingerprint of its claimed origin. A decision limit based on a 99% confidence interval of the reference data is used to define

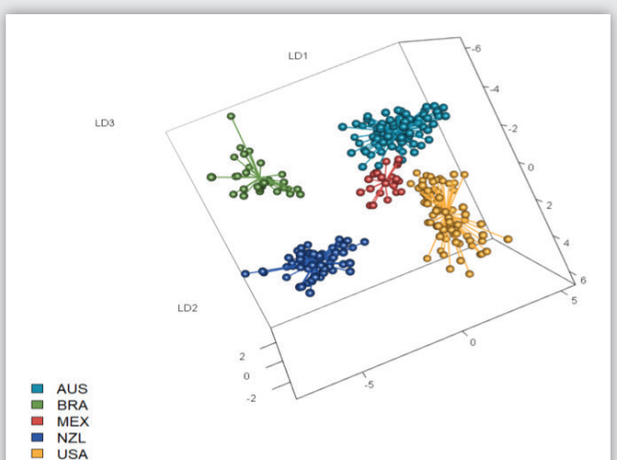

Figure 3. Geographic Identification of serum sources.

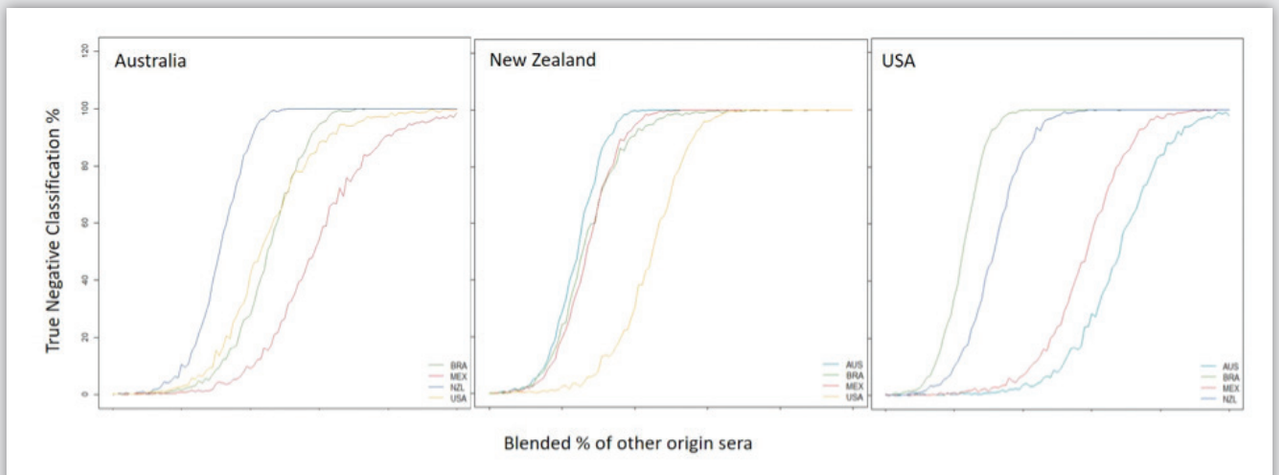

Figure 4. Results of blending experiments.

whether the result is considered 'pass' (consistent) or 'fail' (inconsistent) with the database. At this time, a total of seven geographies can be identified.[12] It should be noted that USA, Mexico and Brazil can be clearly differentiated using this methodology.

Similarly, the results from early experiments (Figure 4) show that mixtures can be recognized, thus providing further information on the source of the material provided. Briefly, blending is more readily detected where it has occurred between origins of distinct fingerprints and to this end, economically motivated levels of blending can be identified.

Further experimentation will be undertaken to refine this data. A detailed publication on this overall approach is being developed.

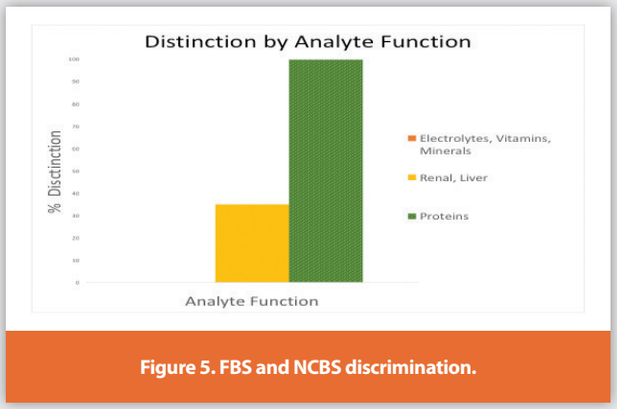

Figure 5. FBS and NCBS discrimination.

Age of Animals

Another area of interest to the industry and its customers is the determination of the age of source animals. The ISIA has worked closely with member companies to support an effort to identify biomarkers of animal age. It has long been suggested that IgG levels could be used to indicate whether serum came from a fetus or a newborn calf.[13] This alone did not give sufficient discrimination to resolve the issue. Previous reports suggested that Gamma Glutamyl Transferase (GGT) was detectable in serum.[14] A veterinary blood chemistry panel was run for known samples of FBS and newborn calf serum (NCBS), and the data was analyzed for the ability of different markers to provide clear differentiation. This indicated that both kidney/liver function and proteins could provide insight into animal age (Figure 5).

On further detailed analysis comparing the 29 tested components, it was determined that a combination of IgG with the liver specific enzyme GGT gave a 50-fold difference in resolution between NBCS and FBS.[15] A detailed publication is currently in preparation. ISIA is working to provide a recommendation with regard to specifications for these two markers, to allow for better characterization of serum based on the age of the source animal.

Gamma Irradiation

While ISIA programs are providing further clarity on the source of FBS, the concern about the possible introduction of adventitious agents remains. ISIA has therefore been working with a panel of experts, suppliers and irradiators to document various aspects of gamma irradiation (Table 2), the current post-manufacturing treatment of choice. This procedure is not well understood by many, and has been the source of much discussion.

The intent has been to develop detailed, scientifically based information on all aspects of the gamma irradiation process. To date three papers have been published:

1. Gamma Irradiation of Animal Serum: An Introduction,[14]
2. Gamma Irradiation of Animal Serum: Validation of Efficacy for Pathogen Reduction[15]

Table 2. Gamma Irradiation panel.

Participant	Affiliation	Representing
Sue Brown	TCS Biosciences	Supplier
Bart Croonenborghs	Sterigenics	Irradiator
James Dunster	Moregate BioTech	Supplier
Debbie Elms	ThermoFisher Scientific	Supplier
Randy Fitzgerald	Proliant	Supplier
Greg Hansen	GE Healthcare	Supplier
Karl Hemmerich	Ageless Processing Technologies	End-user
Huw Hughes	Zoetis	End-user
Robert Klostermann	Merial	End-user
Raymond Nims	RMC Pharmaceutical Solutions	End-user
Mark Plavsic	Torque Therapeutics, Inc	End-user
Andy Pratt	Underwriters laboratories	End-user
Mara Senescu	Steris	Irradiator
Marjorie Van Robays	GSK	End-user
Rosemary Versteegen	ISIA	Industry
Martell Winters	Nelson Laboratories	End-user

3. Gamma Irradiation of Frozen Animal Serum: Dose Mapping for Irradiation Process Validation.[16]

Three further papers are in development focused on:

1. the effect of irradiation on polymers,
2. the requirement for maintenance of the cold chain
3. outstanding issues surrounding irradiation are in development.

Conclusion

The ISIA has undertaken several initiatives to provide increased scientific information in support of risk management, as reviewed above. The Association will continue to develop and expand these to provide clarity and security to end users and regulators.

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Author Biography

After receiving her degrees from the University of Glasgow in Scotland, Dr. Versteegen studied at Cambridge University, England, and the National Institutes of Health, Washington D.C. Subsequent to several years as a staff scientist she joined the biotech start up BRL in 1982. She was with Life Technologies, in all its incarnations, until the Invitrogen acquisition in 2000, holding Vice Presidential positions in areas such as New Business Development, Manufacturing and Regulatory Affairs. After several years as a consultant, she was instrumental in founding the International Serum Industry Association (ISIA) in 2006. She has held the position of CEO since the inception of the Association.