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Volume 18, Open Access (May 2019)

Citation: Versteegen R, Shatova O, Lind S, Linterman K. Testing for geographic origin of fetal bovine serum. BioProcess J, 2019; 18. https://doi.org/10.12665/J18OA.Versteegen. Posted online May 15, 2019.

BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Testing for Geographic Origin of Fetal Bovine Serum

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Abstract

Since its inception in 2006, the International Serum Industry Association (ISIA) has been focused on providing a more informative characterization standard for animal sera. A fundamental aspect of this effort has been the development of a program focused on product traceability from abattoir to end-user. This goal has been achieved in part by implementing the ISIA-sponsored audit program. Serum vendors determined to be compliant with all audit requirements are awarded ISIA Traceability Certifications. In conjunction with Oritain Global Ltd, ISIA has developed and implemented a method for establishing geographical origin of serum products. The method and its capability of determining geographical origin are described in this paper.

Introduction

Serum and other animal-derived products play a critical role in biomedical and biopharmaceutical research, development, and manufacturing. Over the last 60 years, the use of these materials has contributed enormously to the fight against human and animal diseases. 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, however, continued cause for concern. For many years, most regulatory bodies have allowed 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] It has been determined that the alternative animal material-free replacements are also not without their risks. Plant-derived materials, for example, can introduce both animal- and plant-derived adventitious agents into cell cultures.[3] Risk mitigation is expected by national regulatory bodies and can take the form of barrier inactivation treatments such as gamma irradiation.[4] Also required are sourcing strategies that include proper attribution of geographic origin to further the understanding of regional adventitious agent exposure.

Current applications involving the use of animal serum, specifically fetal bovine serum (FBS), can include the production of: legacy products where serum is specified in the manufacturing protocols; primary cells used in the early stages of cellular therapy development; products requiring extensive post-translational modification; and materials where speed to market, or aggressive cost management, is essential. FBS is also used extensively in cell culture focused on basic research and drug discovery. Research users appear to be primarily concerned with both the cost of serum, and achieving consistent cell growth performance using serum without lot-to-lot variabilities.

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 (**Figure 1**).

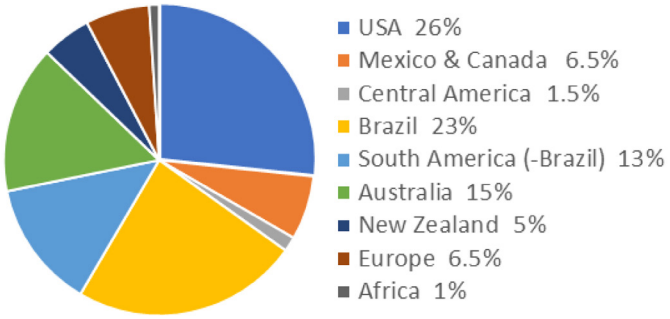

FIGURE 1. Comparison of estimated FBS production by geographical region.

The movement of animal-derived materials between countries is heavily controlled in the interests of animal health, with significant variations in the import regulations existing between different geographies. Biopharmaceutical companies and vaccine producers prefer to use material sourced from countries offering a low risk of infectious disease and a robust infrastructure for animal management and product movement. At this time, the geographies of choice for manufacturers procuring FBS are New Zealand, Australia, and the USA. Supply and demand would suggest that when the most desired material is in relatively short supply (New Zealand only collects about 5–6% of the global supply of FBS) it would be the most expensive, and this holds true. There can be a ten-fold difference in price between South American and New Zealand serum.

Due to the potential for financially-motivated serum

mislabeled products, ISIA has implemented a program to establish and confirm serum authenticity. As a key initial phase, ISIA Traceability Certification has been awarded to those companies that have successfully passed a third-party audit, conducted by an ISIA-approved auditor, according to an ISIA-approved audit plan. Currently, 22 individual companies hold this certification (**Table 1**), while others are working towards this goal.

The significant differences between the ISIA Traceability Certification audit and a routine customer quality audit are the following:

- All records in and associated with the facility under audit can be requested by the ISIA auditor. A company seeking certification must have at least one year's worth of records for the initial approval, which is granted for three years. Subsequent ISIA audits cover all records generated in the prior four years.
- A mass balance analysis is performed at every transfer. This requires access to financial data to determine what and how much product moved, and at what cost, to ensure that what comes in is the same as what goes out.

This type of audit can therefore provide detailed information with regard to the chain of custody of product within the manufacturing process. There are multiple steps in the FBS production process, which are shown in **Figure 2**. From this figure, it is apparent that there are many handoffs between departments or companies required to produce the final product. Each step is subject to audit in the ISIA certification process, as shown in **Table 2**.

The ISIA Traceability Certification program has been a major step forward in assuring the authenticity of animal serum products. The potential for financially motivated mislabeling of sera, as to its geographical source of origin, is such that additional safeguards are required. Traditional

TABLE 1. ISIA Traceability-Certified companies.

Abattoir Basics Company	Life Science Production
Atlantic BioProcessing	Moregate Biotech
Atlas Biologicals, Inc.	Newman Biotech Australia
Axenia BioLogix	Proliant Biologicals
BioArra	River City Biologicals
Biomin Biotecnologia Ltda	River City Biotechnologica Brazil LTDA
BioWest	Rocky Mountain Biologicals, Inc.
By Productos S.A de C.V.	Seratec
Central Biomedia Inc.	Serum Technologies
Corning Inc.	TCS Biosciences Ltd
GE Healthcare	VWR International/Seradigm
Gibco by Thermo Fisher Scientific	

TABLE 2. ISIA Traceability Certification modules. Audit modules D–K address discrete steps in the process.

Module	Scope
A	Auditor and company guidelines
B	Overview
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

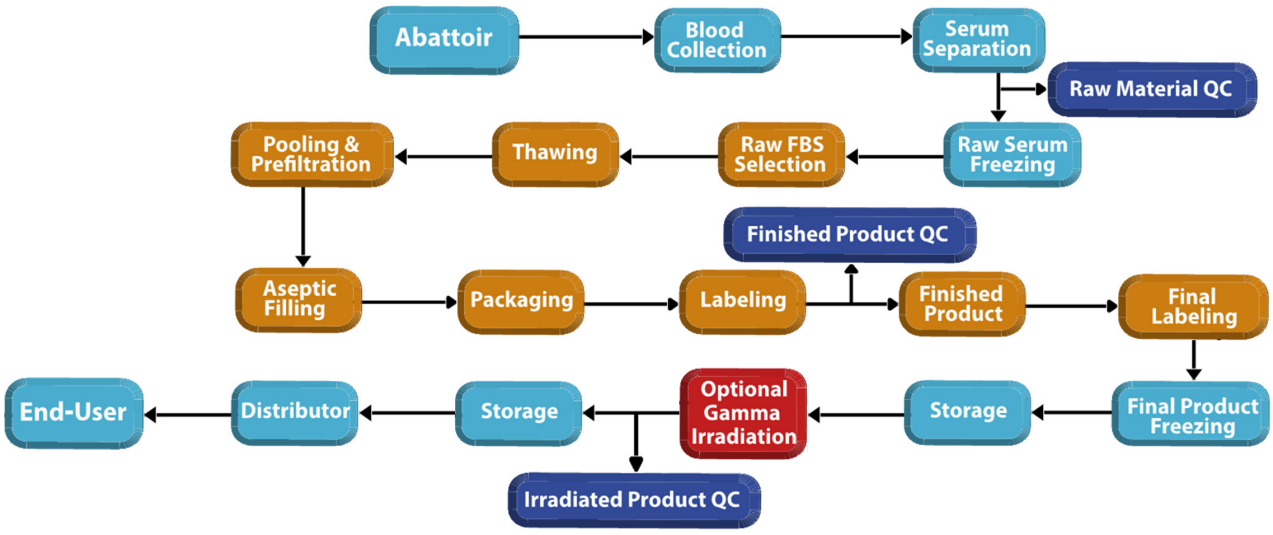

FIGURE 2. Generic steps in the processing of FBS.

types of traceability confirmation rely on packaging and labels, which can themselves be prone to counterfeit. It was recently revealed that South American serum was smuggled into Australia, rebranded as being of Australian origin, and then sold[5]—a painful reminder. Identifying deliberate mislabeling of serum requires specialized methodology. Therefore, ISIA determined that a scientifically based test to identify/verify the geographic origin of a serum product would be necessary to provide additional strength to the traceability program.

This paper describes the establishment of an industry-wide database to support the use of chemical analysis for geographic origin verification, with a primary focus on FBS. This work has been accomplished in partnership with Oritain Global Limited (Oritain), and is based on trace element analysis. This "chemical fingerprinting" relies on differences in the geochemistry of the environment reflected in measured parameters to determine the geographic origin of a product.

Geographic Origin of Serum

ISIA has been researching ways to identify the geographic origin of materials since 2008, having designed pilot experiments using stable isotopes. Widely used and accepted in the food industry, this technique demonstrated that some level of resolution could be attained with serum. However, the inability to differentiate between materials sourced from Texas and Mexico proved to be of concern when differentiating (**Figure 3**).

Further study revealed that chemical profiling using elemental analysis was more applicable to serum. It has been well established that soil geochemistry and climactic patterns differ by geography. Plants take up chemicals from their environment in a ratio consistent with the local geography, forming a chemical fingerprint of the environment in which the plant was grown. Likewise, ruminant animals such as cattle acquire an elemental analysis fingerprint relating to their origin through the consumption of local food and water.

The science used by Oritain originated in the criminal forensic field where it has been used in numerous investigations. The methodology has been thoroughly peer-reviewed and the subject of numerous scientific journal publications over the last 20 years.[6-9] ISIA and Oritain have teamed up to apply this technology to bovine serum authentication. Oritain analyzes serum products through chemical methods and statistical interpretation to determine the geographical origin of the sample. A comparison can be made using sample material acquired at any point in the supply chain. The results are compared to a pre-developed database, supporting or refuting geographical origin claims recorded on traceability documentation.

ISIA and Oritain have built an industry database which can then be used to certify the geographic origin of serum. The serum samples required for this work were donated by traceability-certified ISIA members worldwide. Supplied samples originated from the USA, Canada, New Zealand, Australia, Brazil, and Mexico.

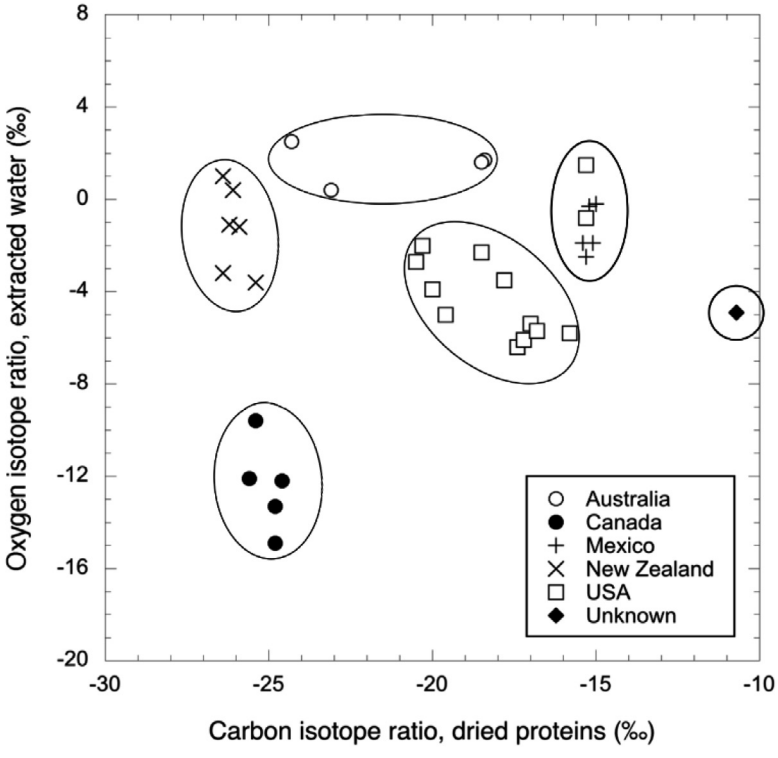

FIGURE 3. Pilot study using stable isotopes to determine geographic origin of serum.

To develop the unique fingerprints of FBS originating from different countries, 2 mL samples of serum were prepared following a standard microwave-assisted digestion methodology (based on the US Environmental Protection Agency [EPA] method 3051A[10]) and analyzed with inductively coupled plasma mass-spectrometry (ICP-MS). The results were corrected for ICP-MS controls, including reagent blanks and digested mass, to determine true elemental concentrations in parts per million (ppm). Data quality was validated at this level using a quality control (QC) material (characterized FBS standard) and a certified reference material (multi-element water). Statistical analysis was carried out on a subset of analyzed elemental concentrations using Oritain's proprietary statistical models.

Statistical data quality was checked for normality, and any outlying datapoints were identified and validated with repeated analysis. Sample set size was also checked using general methods to validate that the subset size was appropriate. Outputs included univariate and multivariate statistical analyses using a combination of general exploratory and discriminatory models.

Figure 4 displays a three-dimensional graphical interpretation of the discrimination model developed to differentiate serum by geographical origin. Each datapoint represents the chemical fingerprint of an individual batch of serum. Simply put, datapoints that cluster together have similar chemical fingerprints and are related by geographical origin, while spatially separate clusters are of distinct geographical origin. The axes denote statistical scores and, in the current context, can be considered arbitrary.

For verifying the geographical origin of serum, a sample tested using this analytical methodology is compared 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 whether the result is considered "pass" (consistent) or "fail" (inconsistent) with the reference database. At this time, a total of eight geographical regions can be identified, and five have been thoroughly characterized. It should be noted that USA, Mexico, and Brazil can be clearly differentiated from each other using this methodology.

Performance of the dataset is evaluated using "leave one out" exhaustive cross-validation as well as benchmarking using samples external to the reference dataset. The results of cross-validation using the model depicted in **Figure 4** are shown in **Table 3** for the five thoroughly characterized geographical regions. Both sensitivity (true positive rate) and specificity (true negative rate) of the test are excellent. True positives are genuine samples that are correctly classified as consistent with the claimed geographical origin. True negatives are non-genuine samples that are correctly classified as inconsistent with the claimed geographical origin. While the model shown in **Figure 4** is the main algorithm used by Oritain, it should be noted that there are several others with similar performance parameters that are used in concert to further minimize errors in testing. In total, twelve independent statistical models can be used to interrogate the chemical fingerprint in relation to geographical origin analysis.

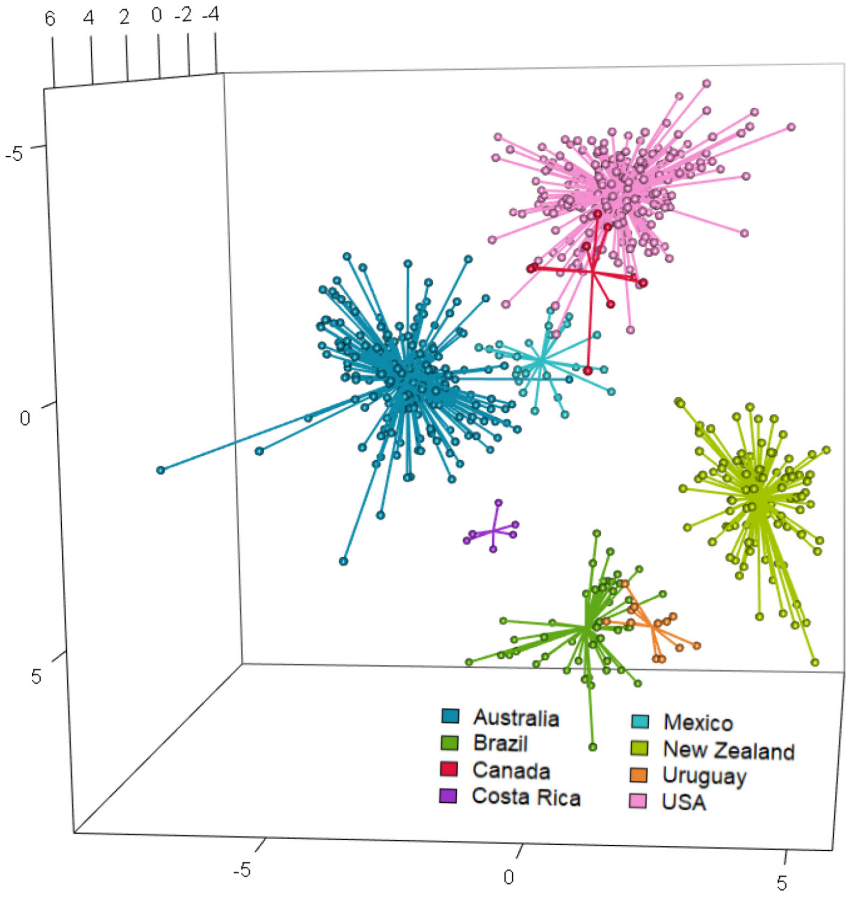

FIGURE 4. Graphical representation of the model used to assess geographical origin of FBS samples. The X, Y, and Z axes represent linear discriminants defined by the LDA model to differentiate the samples.

TABLE 3. Dataset performance rates by geographical origin.

Sensitivity	Australia	Brazil	Mexico	New Zealand	USA
True Positive Rate	>95%	>95%	>95%	>95%	>95%
True Negative Rate	>80%	>80%	>80%	>80%	>80%

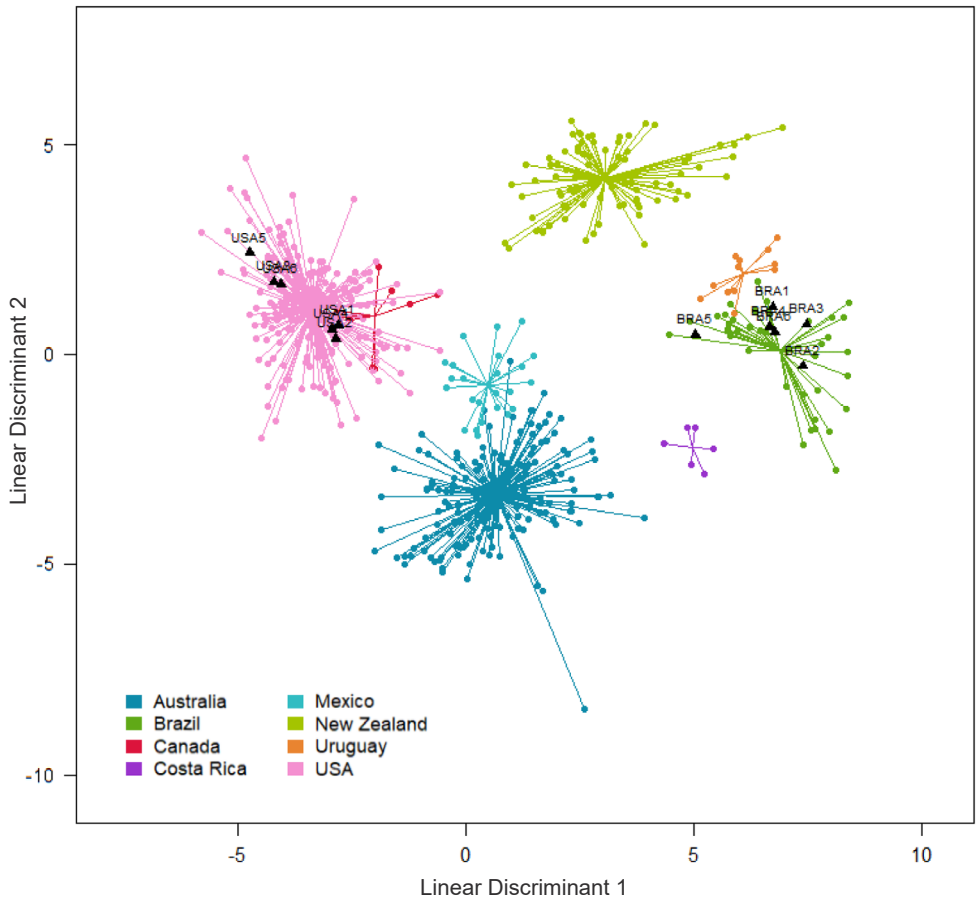

FIGURE 5A. A statistical representation of the geographical origin for blinded samples. Results from the blinded samples are depicted as black triangles.

An example of a pass/fail scenario is displayed in **Figures 5A** and **B**. Blinded samples with the claimed origins of USA and Brazil were tested against the existing database. It can be seen in both graphical representations that the sample from USA is consistent with the statistical representation of the true USA baseline, while the sample from Brazil is not consistent. The reverse is true for the Brazilian samples. These samples would be reported as pass (consistent with origin) and fail (inconsistent with origin) respectively.

The red line shown in **Figure 5B** denotes the 99% confidence interval for the USA fingerprint. By default, 99% of samples truly originating from the USA will fall below the red line. Samples that are farther from the baseline generally have a fingerprint that is more distinct from the mean for the group. This reflects that natural variability between samples and is neither good nor bad.

The chemical fingerprint attributes of "pooled" versus "unpooled" sera have also been studied. During the production process, all of the material collected from one abattoir within a single day is considered a batch. Several

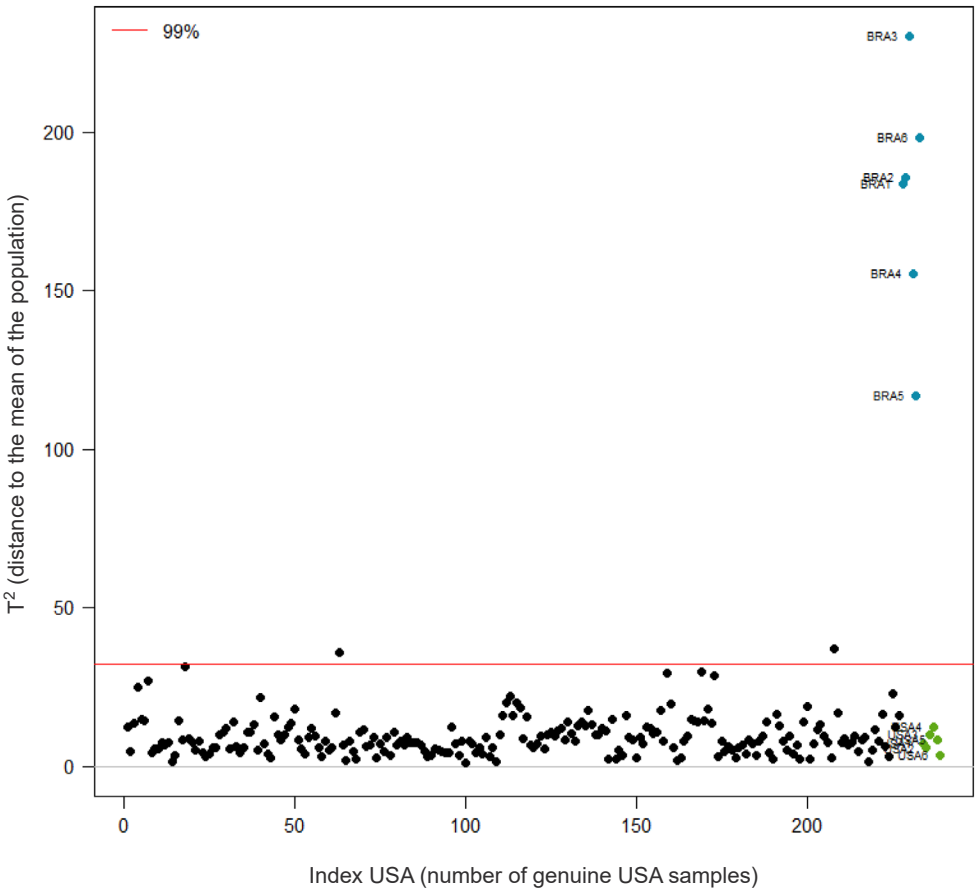

FIGURE 5B. A statistical representation of the determination of geographical origin for USA-derived FBS. Six blinded samples each of USA and Brazilian origin serum are depicted as green (USA) and blue (Brazil) dots.

batches from one country will be pooled to create the starting material for a finished lot of serum (as detailed in **Figure 2**). Such a lot will typically be around 2000 L in volume and can represent thousands of animals from a relatively broad regional distribution. This pooling averages the chemical fingerprint such that the variability of the sample set is significantly reduced, yet the geographically defined origins still remain distinct from one another. **Figure 6** shows that both raw and finished serum can be verified as to geographical origin using the Oritain methodology. Moreover, the fingerprinting approach can be targeted toward samples from every point of the supply chain. This is highly relevant for serum because producers/suppliers are rarely the abattoir owners and have to rely on the veracity of traceability information provided earlier in the supply chain.

The possibility for mixtures (or "blends") of serum from differing geographical regions is another area of concern. Such blending can be recognized using chemical fingerprinting, thus providing further information on the source of the material provided. For major production regions, adulteration by blending can be identified to a certain degree, as shown in **Figure 7** (on the following page).

To create a detection example, blended serum samples of USA:Mexico and USA:Brazil origins were intentionally created at various ratios. These blends were analyzed and interpreted using typical Oritain procedures. **Figure 7A** illustrates that USA blends falling outside of the USA fingerprint cloud are drawn toward the adulterant origins of Brazil or Mexico. Furthermore, blended samples clearly fall outside the 99% confidence interval of the genuine USA fingerprint (**Figure 7B**), and the higher the proportion of the blended serum from a non-genuine origin, the further from the baseline the sample is statistically and visually positioned.

A wide series of blending studies (data not shown) has explored the capability of this methodology for this aspect

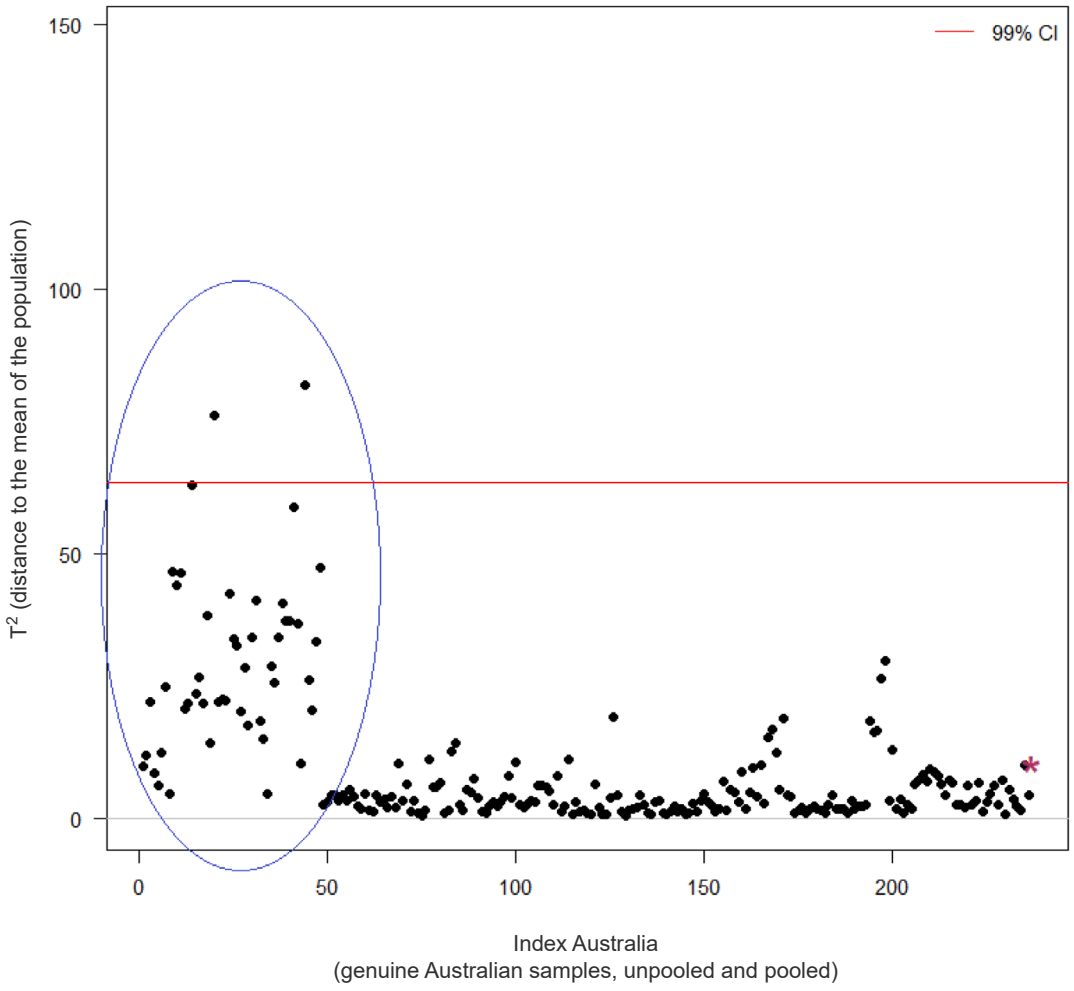

FIGURE 6. A graphical representation of the results from Australian FBS samples contained in the database. Each black dot corresponds to an individual sample. Samples included in the blue circle are regional (Australian) unpooled samples, while samples outside of the blue circle are pooled samples. The red star is an example of a sample being tested against the database.

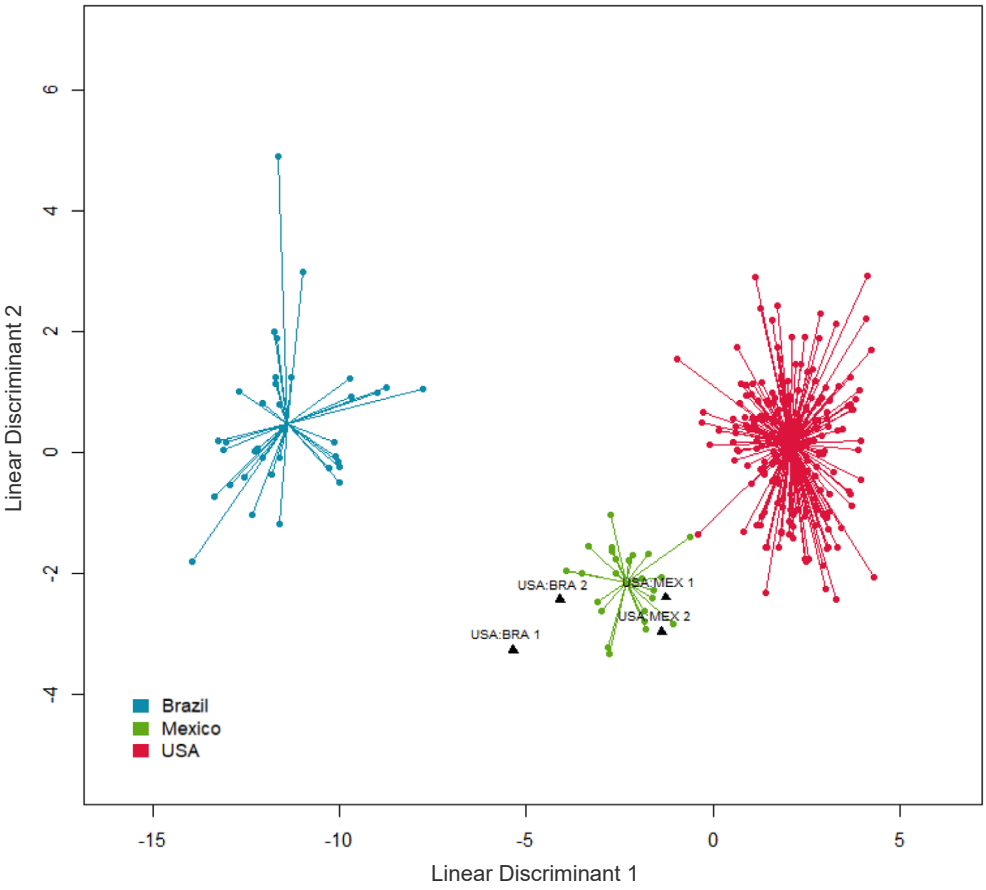

Figure 7A. A statistical determination of geographical origin for the USA-derived FBS. USA blends fall outside of the USA fingerprint cloud and are drawn toward the adulterant origins of Brazil or Mexico. USA:Brazil and USA:Mexico serum blends are depicted as black triangles.

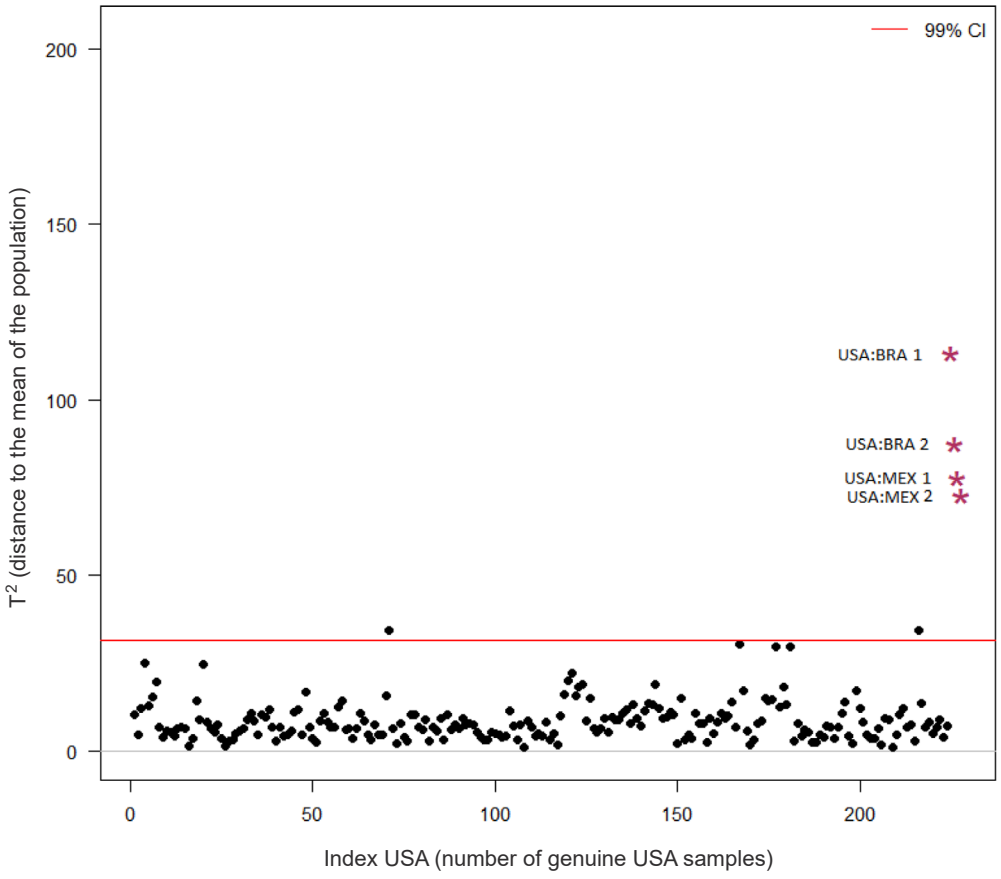

Figure 7B. A statistical determination of geographical origin for the USA-derived FBS. The higher the proportion of blended serum from a non-genuine origin, the further from the baseline the sample is statistically and visually positioned. USA:Brazil and USA:Mexico serum blends are depicted as red stars.

of possible serum adulteration. It is not surprising that blended samples and serum origins indicating noticeable distinctions from each other are more readily identified. Likewise, a sample with a higher percentage of blending has a higher likelihood of detection than a lower blending rate. Based on the current dataset and industry knowledge, it can be concluded that a higher-priced serum product, blended but not disclosed, can be readily identified.

Conclusion

The chemical fingerprinting approach described in this paper has proven to be applicable to a wide variety of commodities. It is widely employed in the food and textile space currently.[11-16] Fingerprinting of FBS was described above. Bovine calf and adult serum have also been investigated (data not shown). These products can be geographically distinguished with chemical fingerprinting, as with FBS, but each also retains a distinct fingerprint, allowing different categories of serum to be differentiated from each other. It is a natural progression to envisage successful deployment of a wider array of biological products for diagnostic/health use. With this development, the ISIA continues its commitment to providing a more informative characterization standard for animal sera.

Acknowledgments

Thanks are due to Thermo Fisher Scientific, GE Healthcare, Animal Technologies, Axenia BioLogix, VWR International/Seradigm, Biomin Biotecnologia Ltda, By Productos S.A de C.V., Life Science Production, Moregate Biotech, and Vacca Biologics (Corning) for contributing serum samples.

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