



WHITEPAPER

USING CHEMICAL FINGERPRINTING TO VERIFY THE ORIGIN OF FETAL BOVINE SERUM

Using chemical fingerprinting to verify the origin of fetal bovine serum

Having confidence in country of origin of sera is of utmost importance to vaccine and pharmaceutical manufacturers. GE Healthcare therefore has collaborated with Oritain™ to align on an independent test-based traceability program to provide proof of the country of origin by testing actual serum samples to supplement the use of on paper documents. Through the use of analytical chemistry and statistical interpretation, Oritain has developed a service to verify the fetal bovine serum origin based on the chemical fingerprint information that innately exists within the product. This paper presents the use of Oritain's serum origin technology to scientifically verify provenance of fetal bovine serum (FBS) for GE Healthcare.

Introduction

Many existing and new-generation protein drugs and vaccines required for human health and veterinary purposes rely on a cell culture manufacturing process. Whereas biological drugs are mostly produced under serum-free conditions, industrial vaccine production often requires FBS or serum derivatives. FBS is also used for cell culture in basic research and drug discovery. Whereas research users are largely concerned with the consistency of cell growth performance of serum from lot to lot, users in the industrial vaccine production segment are more interested in regulatory conformance and the lack of adventitious agents (1). Moving away from serum in general would be the best option for vaccine manufacturers, but it is not practical from an economic or regulatory perspective for most legacy vaccines. In addition, there are tens of thousands of different components in serum, whereas the most complex chemically defined media have between 100 and 150 components. Even today, the interactions between all of the serum components are not well understood, and for some cell lines, nothing performs as well as serum.

No matter what country it originates from, FBS will fortify cell culture media and support cell growth in a very similar manner. However, the variability between different serum batches remains a concern for the user. There is as much variability between serum batches within a country of origin as there is between countries of origin. For researchers, this is good news from a supply standpoint, as they can select the cheapest serum that can reliably reproduce results. The country of origin of the serum is of less importance. In contrast, for the vaccine manufacturers, the safety of the serum is of number one priority. Because sera are derived from animals, the presence of virus in serum is unavoidable, and different countries of origin present different levels of risk of specific virus contamination (2). Whereas certain bovine viruses occur worldwide, other bovine diseases display distinct geographic prevalence. Regarding bovine spongiform encephalopathy (BSE), for example, FBS from New Zealand and Australia has been promoted as “safer” than FBS of other origins, as these countries have isolated cattle herds and have never reported an outbreak. However, BSE has now been determined not to be transmitted in bovine blood or blood products, when appropriate slaughter practices are adhered to (2, 3).

The World Organization for Animal Health (Office International des Epizooties) is an international organization that monitors and provides updates on the presence of disease in different countries. As of January 2017, New Zealand, Australia, and the USA presented the lowest level of risk for infectious agent contamination in serum, including BSE, transmissible spongiform encephalopathy (TSE), and foot and mouth disease (FMD). For this reason, many vaccine manufacturers have one or more of these countries of origin specified in their manufacturing protocols that are registered with the FDA or international equivalents. These manufacturers have high demands on their serum suppliers to ensure that the country of origin is not only procuring accuracy from a regulatory standpoint, but also from an infectious agent standpoint (4).

While there are further steps that vaccine manufacturers can take to limit virus contamination risk, including gamma irradiation of the serum, these do not necessarily eliminate all risk of live virus being present in the serum. In fact, recent evidence suggests that some small non-enveloped viruses are not impacted by gamma irradiation (5). Whether these viruses are of concern is, however, an area of debate. In addition, some cell lines do not perform as well with irradiated serum, because gamma irradiation can have a negative impact on proteins present in the serum.

As a consequence of the large demand of sera from low-risk countries, there is a significant price premium paid for FBS from these origins. Today, Australian FBS, for example, is roughly nine times more expensive than Brazilian FBS. In addition to being driven by demand, prices for FBS are also driven by regulations and serum availability (1). Import-export regulations limit which sera can be brought into different countries. For example, under current regulatory conditions, Brazilian serum can be imported into the European Union and most Asian countries, but is barred from being imported into the USA. Moreover, as serum is a by-product of the meat industry, weather and other macro-economic factors impacting the beef industry can impact availability of FBS separately from the impact of demand. Limited supply in the USA and Australia recently drove those sera to record high pricing (Fig 1).

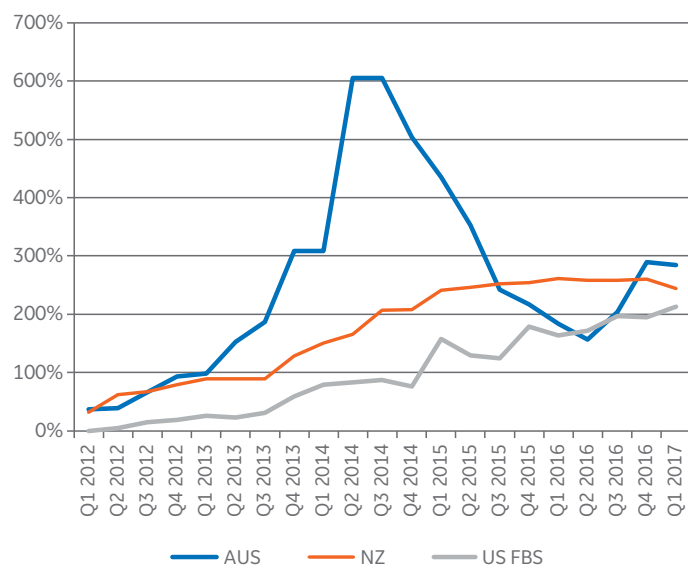


Fig 1. Relative cost (percent increase) of raw serum from Australia, New Zealand, and the USA between 2012 and 2017.

With the large price discrepancies between countries of origin, there is a financial incentive to cheat. By simply replacing the word Brazil on a bottle with the word Australia, the serum is instantly worth nine times more. From a customer standpoint, there is no way to tell the difference, as both Brazilian serum and Australian serum will have similar cell growth characteristics. However, the risk of virus contamination is different for the two products. Serum manufacturers and suppliers have a moral obligation to ensure that the country of origin is 100% accurate, and vaccine manufacturers are paying a substantial premium for low risk countries of origin to ensure that their vaccines that eventually end up in human beings have the lowest risk possible of doing harm.

The serum industry is largely self-regulated, and the International Serum Industry Association (ISIA) was not established until 2006. The ISIA has made major strides in standardizing terminology within the industry and establishing an independently audited traceability certification process that companies can qualify for. This traceability program has been very successful in curtailing the amount of fraudulent sera in the market place. Nevertheless, like most paper based traceability systems, it is limited in that someone does not willingly falsify documents.

Previously, when geographic origin could not be verified by testing, the buyer was left to rely only upon documentation for verification of origin. However, this has changed and traceability testing of animal products is now possible. Therefore, GE Healthcare decided to take the verification of serum origin one step further and has joined forces with Oritain as the provider, to introduce an independent traceability testing program based on Oritain's proprietary scientific fingerprinting technology.

The tests are now performed to confirm country of origin of all lots of characterized and defined FBS from Australia, New Zealand, and the USA for GE Healthcare. This program supplements the ISIA certification and the use of paper documents as an additional assurance of the integrity and authenticity of the FBS.

How chemical fingerprinting works

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 (Fig 2).

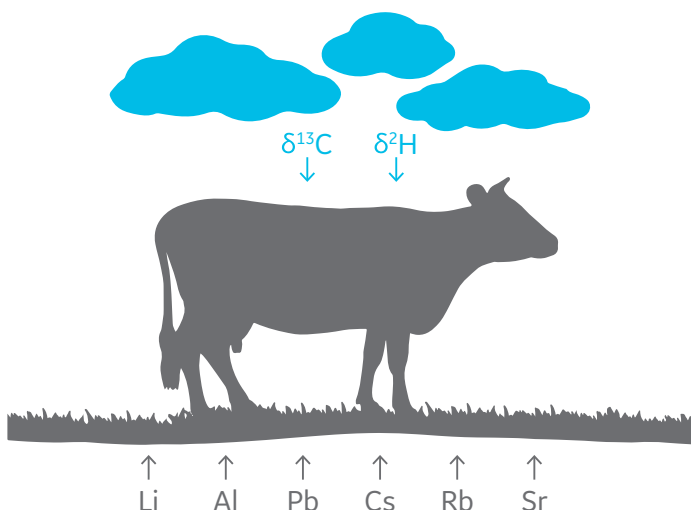


Fig 2. Chemical fingerprints are based on chemical uptake from the environment.

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

Developing a method for chemical fingerprinting of sera

To develop the unique fingerprints of sera from different countries, GE provided samples from individual lots of HyClone™ FBS from four different countries of origin (Table 1). A 2 mL aliquot of each FBS sample was pipetted into a cleaned polytetrafluoroethylene (PTFE) digestion tube (exact sample mass was recorded) and 5 mL of quartz distilled HNO₃ was added to the tube, which was then capped for a 1 h pre-digestion. Pressure was released from the tubes and samples were microwave-digested for 1 h. The digest was transferred to a new tube (and rinsed out with

deionized water) and the solution evaporated to dryness in a graphite hot block. Thereafter, samples were re-dissolved in 10 mL of 2% HNO₃ and sent for trace element analysis via inductively coupled plasma mass spectrometry (ICP-MS).

Table 1. Number of samples of FBS tested from each country of origin

Country of origin	Total sample no.
Australia	47
Brazil	10
New Zealand	22
USA	33

Results were corrected for blanks and digested mass to determine true elemental concentrations in ppm. Data quality was validated at this level using a quality control (QC) material (characterized FBS standard) and a certified reference material (CRM, multielement water). Statistical analysis was 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 were identified and validated with analysis repeats. Sample set size was also checked with 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.

Differentiation of sera from different origins

Figure 3 displays the graphical interpretation of the discriminant model developed to differentiate serum origins. Each data point represents the chemical fingerprint of an individual batch of serum. Most simply, points that cluster together have similar chemical fingerprints and are related in origin, while clusters that are separate from each other are of distinct origins. The values on the axis can be considered arbitrary but denote the variability of the chemical fingerprints.

Verifying the origin of HyClone FBS

The described model was also used to verify serum of a claimed origin. Baseline fingerprints of a number of HyClone FBS production lots were used to define a distribution for the distance of samples from the centroid of the multivariate cluster for a particular origin. Figure 4 shows a simulated example of a passed (origin: New Zealand) and a failed (origin: Brazil) result for a claimed origin of New Zealand. The reference fingerprint of the New Zealand samples are depicted as gray dots, with a 99% confidence interval of this data denoting the decision limit (blue line) between tested samples considered 'consistent' or 'inconsistent' (pass/fail) with the reference origin.

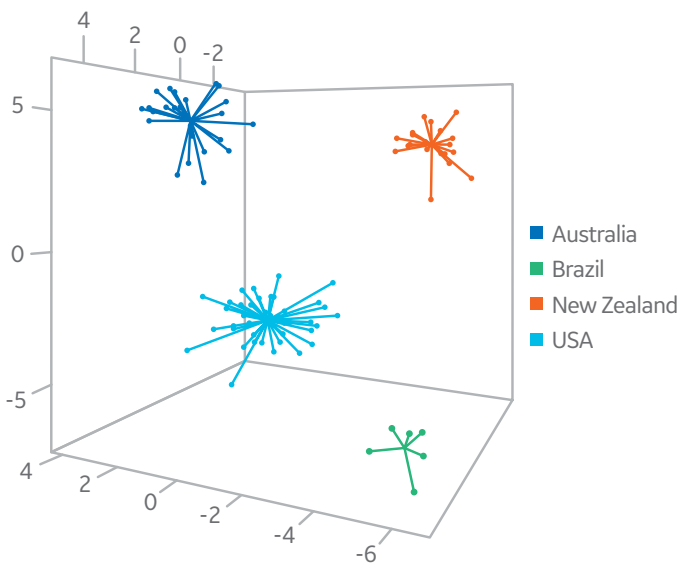


Fig 3. Graphical representation of the model used to assess serum sample origins.

Importantly, the data clusters did not overlap each other across these three dimensions. Overall, this model performed well in terms of the ability to differentiate sera from four different origin groups based on their chemical fingerprints. Note, that samples sourced from Brazil, (high-volume, low-value origin) were easily discernible from the samples sourced from the other high-value origins. Overall performance is exemplified in the correct classification rate data in Table 2. As displayed, the false positive/negative rates are good (< 2% in most cases). Although this model is the main algorithm used by Oritain, there are several others, with similar performance parameters, that are used in concert to further minimize errors in commercial programs. In total, 12 independent statistical models are used by Oritain to interrogate the chemical fingerprint in relation to origin analysis.

Table 2. Model performance indicators

	AUS	BRA	NZL	USA
Sensitivity	98%	100%	100%	97%
Specificity	98%	100%	100%	99%
False positive rate	2%	0%	0%	1%
False negative rate	2%	0%	0%	3%
# of samples	47	10	22	33

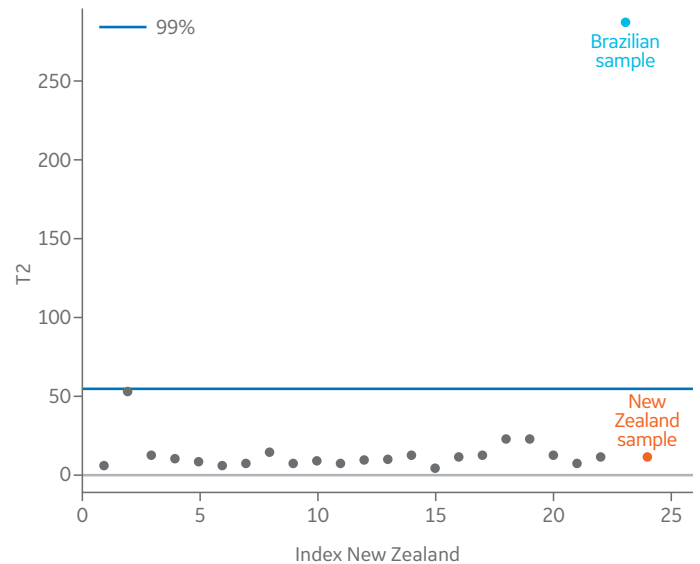


Fig 4. Simulated audit of claimed New Zealand origin FBS. The x-axis shows count of samples, and the y-axis depicts the distance from the 'multivariate mean' (all chemical components measured), of the New Zealand reference data. Reference samples are shown in gray. A true New Zealand origin test sample is shown in red and a "fraudulent" sample (origin: Brazil) is presented in light blue.

The effect of "origin blending" has also been investigated with binary mixes of differing origins. Initial studies show that it is also possible to identify "economic" levels of blending using this method (results not shown).

A decision limit of a 99% confidence interval (blue line) is used to define the pass/fail limit for a tested sample.

Conclusion

Here, we describe the development of Oritain's technology to identify the origin of sera. Using Oritain's proprietary statistical algorithm, authenticity of a claimed serum origin can be verified and a fraudulently claimed origin identified. With the help of Oritain's service, GE Healthcare takes a lead in the industry to chemically verify serum provenance and remains committed to the ISIA to roll out a larger industry wide-test based traceability program.

Contributors

Sam J. Lind (Ph.D., Science Commercialisation Director, Oritain), Robert Curry (MBA, Senior Product Manager for Serum, GE Healthcare), Cecilia Anneren (Ph.D., Global Product Marketing Manager, GE Healthcare), Cory Card (Principal Scientist, GE Healthcare), and Kiri McComb (Ph.D. Chief Technical Officer, Oritain).

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GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA

GE Healthcare Dharmacon Inc., 2650 Crescent Dr, Lafayette, CO 80026, USA

HyClone Laboratories Inc., 925 W 1800 S, Logan, UT 84321, USA

GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden

For local office contact information, visit gelifesciences.com/contact.

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