

### ***What is “bovine serum”?***

Serum is the centrifuged fluid component of either clotted or defibrinated whole blood. Bovine serum comes from blood taken from domestic cattle. Serum from other animals is also collected and processed but bovine serum is processed in the greatest volume.

Whilst the procedure of making serum may seem to be straightforward, the processing of serum takes place under very tightly controlled conditions. The process has been carefully developed and uses sophisticated facilities and equipment, and is accompanied by extensive testing. The levels of control and testing are particularly stringent when processed bovine serum is intended for use in the production of medicinal products.

## ***Where does it come from?***

Bovine serum is a by-product of the meat industry. Bovine blood may be taken at the time of slaughter, from adult cattle, calves, very young calves or (when cows that are slaughtered are subsequently found to be pregnant) from bovine fetuses. It is also obtained from what are called "donor" animals, which give blood more than once.

Blood is available from bovine fetuses only because a proportion of female animals that are slaughtered for meat for human consumption are found (often unexpectedly) to be pregnant. Blood is available from very young calves because calves, especially males from dairy breeds, are often slaughtered soon, but not necessarily immediately, after birth because raising them will not be economically beneficial. Older animals are, of course, slaughtered for meat.

Only donor cattle are raised for the purpose of blood donation. Donor cattle are invariably kept in specialized, controlled herds. Blood is taken from these animals in a very similar way to that used for human blood donation.

Irrespective of whether blood is taken at slaughter or from donors, the age of the animal is an important consideration because it impacts the characteristics of the serum.

Bovine serum is categorised according to the age of the animal from which the blood was collected as follows:

- **"Fetal bovine serum"** comes from fetuses
- **"Newborn calf serum"** comes from calves less than three weeks old
- **"Calf serum"** comes from calves aged between three weeks and 12 months
- **"Adult bovine serum"** comes from cattle older than 12 months

Serum processed from donor blood is termed **"donor bovine serum"**. Donor animals can be up to three years old.

## ***What is bovine serum used for?***

There is a wide range of applications for processed bovine serum. Perhaps the most important is in the field of pharmaceuticals where it is used in the research, manufacture and control of human and veterinary vaccines and of drugs (“biopharmaceuticals”) derived using “biotechnology” (in other words techniques involving living organisms) many of which are at the cutting edge of drug development. Fetal bovine serum is also used extensively in research. A technique known as “cell culture” is widely applied in the manufacture of both vaccines and biopharmaceuticals and bovine serum is extensively used in cell culture. A little more background on cell culture may be of interest here.

Since the middle of the last century there have been many developments in the fields of vaccinology and biotechnology and it would be courting controversy to try to rate them in order of importance. No one, however, would argue that as far as human and animal health is concerned one of the front-runners would be the development of the techniques of cell culture.

Cell culture is the process by which cells – human, animal or even insect – are grown under controlled conditions *in vitro* (outside the body). The possibility of maintaining animal tissues outside the body – at least for short periods of time – was demonstrated more than one hundred years ago. However, it was not until the 1940s and 1950s that the techniques were refined to produce cell cultures that could be used to cultivate viruses (which will only grow in living cells). This paved the way for the first virus vaccine to be produced using cell culture – polio vaccine, in 1955. The contribution to human and animal health of the vaccines that have resulted from the application of this technology is an ongoing and global success story.

More recently cell culture has been combined with what are called recombinant techniques to develop so-called recombinant-derived products (“biopharmaceuticals”). Whereas vaccines are almost invariably used to prevent disease these biopharmaceuticals offer important new opportunities for treatment and diagnosis. They are a hugely important and very rapidly expanding area of modern medicine.

Another significant and growing application of cell culture is in the safety testing of widely used products such as cosmetics and household chemicals. As the use of live animals for the safety testing of such products (cosmetics especially) has come increasingly under the political and ethical spotlight, cell culture technology has been used more and more to reduce or eliminate testing in animals.

These are only three examples of the many available. Cell culture techniques are an essential tool, and have made, and will continue to make, a very significant contribution in many of those areas of biomedical science and research that will benefit humans and animals.

### ***Why is bovine serum important?***

Maintaining cells *in vitro* in a healthy condition and over time is a complex task. They will only survive, grow and multiply if they are well fed and provided with an appropriate and protective environment. Complex mixtures of substances (“media”) are used to bathe the cells in order to both feed and protect them. Different cells have different requirements. In many instances the presence of serum in the mixture is essential if the cells are to grow adequately and normally. Bovine serum is much the most widely used, because high quality bovine serum is available in sufficient volume and has been found to support cell growth very well indeed

### ***Why do cell cultures need serum?***

The short answer is that it both nourishes and protects the cells in a way that almost nothing else has been found to do. Bovine serum stimulates the cells to grow and multiply and helps to keep the cells normal and healthy over time.

The largest proportion of bovine serum used to support the growth of cells in cell culture is fetal bovine serum. It fulfils the function especially well because it contains particularly high levels of substances that promote cell growth. It also has favorably low levels of certain other molecules, including immunoglobulins, which are found in the blood of older animals and may interfere with production processes.

It is the incredible complexity of its molecular make up that makes serum unique – and so valuable.

## ***How is bovine serum produced and tested?***

Bovine blood that is to be processed to serum may be collected either from animals at the time of slaughter or from donor animals.

Fetal bovine blood and blood from animals that are kept in donor herds (see also: *What is bovine serum?*) is collected aseptically (i.e. using equipment designed to protect the material from microbial contamination) – just as is the case with human blood donation. These procedures are carefully monitored. The collection of fetal blood takes place in a specially provided area in the slaughterhouse/abattoir. Blood taken at slaughter from calves or adult cattle is collected under clean, controlled conditions.

The degree to which bovine serum is processed and tested depends to a significant extent upon the use to which it is to be put. What is vital, and is a commitment of the member companies of the ISIA, is that the sourcing, processing and testing are correctly performed, monitored and recorded and the serum consistently meets all relevant rules and regulations and the specific requirements of the customer.

Fetal bovine serum and (to a lesser extent) donor bovine serum are almost invariably used when serum is required for the manufacture of human pharmaceutical products. Veterinary vaccines, however, are often produced using other types of animal serum. Medicinal products must be produced following what is known as “Good Manufacturing Practices” (GMPs), a complex set of rules and requirements that are in place to ensure that the final product will be of the highest possible quality. GMPs impose obligations with regard to the raw materials that are used, like bovine serum, and these must be respected.

Let us take the manipulation of fetal bovine serum and donor bovine serum, destined for use in the manufacture of a pharmaceutical product, as examples of processing and testing.

The aseptically collected fetal or donor blood is refrigerated immediately after collection until coagulation is complete. The blood is then centrifuged to separate the serum from the blood clot and the serum is removed. The resultant serum is kept frozen in carefully identified clean, hygienic containers until it is thawed immediately prior to further processing. Serum, like many materials of biological origin, will deteriorate over time if it is not properly stored. In order to retain its properties it is stored and transported frozen. Great care is taken to ensure that the proper storage and transport conditions are maintained at all times.

A pre-defined quantity of serum is processed and tested at one time to form what is called a “batch” or “lot” of processed product. Processing of each batch is performed under controlled conditions by first thawing the designated material followed by “sterile filtration” which involves passing the material through a series of membrane filters with tiny pores that are of reducing size, the last of which will have a pore size that is small enough to remove and retain bacteria and fungi so that the serum is “sterile” (i.e. all bacteria and fungi have been removed). The batch is frozen again immediately after filtration to preserve its quality.

Samples are comprehensively and carefully tested using approved tests (see also “*Is serum safe?*”) to confirm freedom from bacteria and other microorganisms. Extensive biochemical and biological testing are also performed. One important example: as certain microorganisms produce toxins that could be harmful and that would not be removed by filtration, the processed serum is carefully tested for such substances.

Viruses are not removed by sterile filtration. For this reason the batch must be tested to make sure that it is free from potentially contaminating viruses. As an additional safeguard (but not a replacement for testing) fetal bovine and donor serum is often gamma irradiated (gamma irradiation inactivates viruses) using controlled procedures in facilities specially validated for this purpose. Sterilization of the finished product by heat is not an option, as it would destroy many of the biologically active moieties necessary for cell growth.

Records are kept to ensure that each process step and the results of all testing performed can be linked directly to each individual batch of serum that is supplied to the customer. The customer may also perform tests on the batch prior to purchase and often these tests will be extensive. They are likely to include checks to confirm that the batch will perform optimally by promoting good growth of the customer’s specific cell lines of interest.

The overall testing process is very labour intensive and may take several months to complete.

## ***Is bovine serum safe?***

Bovine serum has a long (now several decades) and extensive history of safe use in the manufacture of human and veterinary pharmaceutical products and in other applications as well.

To describe all of the precautions taken to achieve and ensure this would require a book. What follows, therefore, is only a very brief overview of the key precautions taken to reduce possible risks to a negligible level – keeping in mind especially its use in pharmaceutical manufacture for the benefit of human and animal health.

The conditions and methods of slaughter, collection, storage, manipulation, processing, treatment, testing and transport of the material are all very carefully controlled to keep to a minimum the risk of the serum being contaminated with living microorganisms or undesirable non-living material. (see also: *How is bovine serum produced and tested?*).

The geographical origin of the material is also very important. Bovine blood is only collected in countries that have a known, well-monitored and documented and acceptable (to regulatory authorities) animal health status. ISIA member companies and regulatory authorities make use of the information published by the World Organisation for Animal Health (the “OIE”) in reaching a decision as to which countries are acceptable. The OIE (an intergovernmental organisation) is responsible for establishing and publishing the animal health status of countries/regions. The diseases monitored by OIE include many diseases that are transmitted from animal to animal (e.g. foot and mouth disease) and also potentially zoonotic diseases (i.e. transmissible from animals to man). The principal source countries/regions for serum are Australia, New Zealand and North, Central and South America.

Disease status of a country is not the sole consideration. With the exception of blood from donor animals in donor herds, blood is obtained from animals at the time of slaughter (see also: *What is bovine serum?*). For this reason source countries must have in place an infrastructure that includes authority-licensed slaughterhouses/abattoirs, acceptable to national and international bodies and dedicated to the processing of healthy animals. The animals must be inspected by qualified, authority recognised experts both before and after slaughter and meat from the animals must be passed as fit for human consumption.

In the case of donor blood the donor herds are very carefully controlled, and the health status of the animals is extensively monitored and documented.

The conditions of processing are also critical. ISIA member companies ensure that the production processes are very carefully controlled and monitored. This includes any processes that may need to be contracted out (e.g. gamma irradiation which can only be done in a specialised and licensed facility).

Quality Assurance systems for the monitoring and evaluation of the equipment, facilities and procedures and competencies and training of staff are in place. Companies will perform periodical self-audits as part of quality assurance. They are also subject to frequent audits by authorities and by customers.

Quality control testing is performed in order to ensure that serum products consistently conform to all relevant rules and regulations and meet customer requirements and specifications. Many of the quality control tests that are performed use protocols that have been developed by, or are recognised by, national or international regulatory bodies. When such recognised test protocols exist they are always followed. The tests that are performed include tests that are specifically designed to detect the presence of a very wide range of bacteria, viruses and other microorganisms.

All of the above is comprehensively documented to ensure that procedures can be seen to have been followed and that relevant test results are available for every step and for every batch of processed serum that is produced. The “traceability” of all of the material that makes up each batch of serum from origin to end product is recognised by ISIA member companies as being an imperative; it provides the necessary assurances that a specified batch of serum cannot have been substituted with another of unknown quality (and thus unknown safety). With this in mind, the ISIA has itself established a very comprehensive traceability policy.

## ***What about bovine spongiform encephalopathy?***

Bovine spongiform encephalopathy (BSE, “Mad Cow Disease”) has been very much under the spotlight in recent years. Because humans are susceptible to BSE, one of the questions that is rightly asked is: “*What precautions are in place to prevent the transmission of this disease via medicinal products that have been manufactured using bovine serum?*” A similar question can of course be posed for veterinary medicinal products that are administered to susceptible species such as cattle, sheep and goats.

Importantly, there is no evidence that BSE has ever been transmitted via a medicinal product and experts (including the World Health Organisation) have documented that no infectivity has been found in the blood of afflicted cattle. Of course, precautions still need to be taken, but it is nevertheless reassuring information.

Although BSE is a relatively new disease, similar diseases called transmissible spongiform encephalopathies (TSEs) have been known for a long time in both animals and man. The exact nature of the agents that cause these TSEs is still a matter of scientific dispute, but they are very resistant to many of the chemical and physical treatments that efficiently destroy bacteria, viruses and other micro-organisms. In practice, it is not possible to effectively “treat” a material like bovine serum to inactivate the BSE agent, so precautions must be taken to ensure that the material is free from the BSE agent at the outset and that it is manipulated in such a way as to avoid its introduction. Safe sourcing, safe collection and safe processing in a protected environment are thus of great importance.

The OIE (see also: *Is bovine serum safe?*) monitors the incidence of BSE in the world and publishes this information on a regular basis. Regulatory authorities and processors use it as one of the critical determinants in deciding which countries may be used as a source of serum. Serum for biopharmaceutical use is only derived from countries that are deemed to be acceptable by regulatory authorities and their expert advisers.

BSE is a disease of older animals. This gives rise to an additional safeguard. Source animals for bovine serum must be less than three years old – a cut-off that is based upon scientific input and consensus.

## ***What about rules and regulations?***

The rules and regulations that processors of bovine serum must respect are complex and are frequently changing. It is only possible to provide a brief overview here.

Some regulations are targeted directly towards processed bovine serum that is offered for sale and supplied by serum producers. These, or similar regulations, apply to other materials of animal origin as well. They address the possible risk that serum (or other products) might transfer a disease from one country or region to another that was previously free from that disease. They are in place to ensure that the movement of the products from one country to another, and their subsequent manipulation, does not pose a risk to animal or human health.

Additional requirements become applicable when bovine serum, or other materials of animal origin, are used in the manufacture of products such as pharmaceuticals that are themselves subject to regulation. These rules are in place to ensure that the material can safely be used in its intended application.

Thus this regulatory framework is not unique to processed bovine serum. It applies to many other animal-derived materials as well.

### **Movement**

Export and import of animal by-products such as bovine serum necessarily involves collaboration between different national and international regulatory bodies. Information on the animal health status of countries/regions provided by the OIE (see also: *Is bovine serum safe?*) forms the basis upon which decisions relating to the movement of animal by-products are made.

Veterinary controls worldwide are to a great extent based upon the rules and regulations established by the United States Department of Agriculture (the USDA) and by the European Commission – the two largest global markets for animal-derived products being the USA and Europe.

### **Use in the manufacture of human and animal medicinal products**

Serum (together with other animal-derived materials) destined for use as a raw material in the manufacture of medicinal products is, necessarily, subject to particularly stringent additional requirements relating to quality and safety. Numerous regulatory bodies worldwide are involved in setting standards and defining requirements that impact upon bovine serum either directly or indirectly.

In the USA these are the USDA, the Food and Drug Administration (FDA) and the United States Pharmacopoeia.

Both the USDA and the FDA have in place regulations that address in detail the control of bovine serum that is to be used in the manufacture of medicinal products. With regard to BSE, the USDA maintains a list of countries from which bovine serum cannot be derived in order to minimise any risk. The FDA also applies the same geographical restrictions.

In Europe the bodies are the European Commission, the European Directorate for the Quality of Medicines and Healthcare (the EDQM – an organisation responsible, amongst other things, for the

European Pharmacopoeia) and the European Medicines Agency (the EMA – which reports to the European Commission and is responsible, with the support of national authorities, for the scientific evaluation of medicines for use within the EU).

These bodies in turn make use of scientific advice issued by supra-national organisations such as the World Health Organisation (WHO) and the World Organisation for Animal Health (OIE).

One of the responsibilities of the EMA is to produce scientific guidelines that cover a whole host of matters relating to the development, manufacture and control of human and veterinary medicinal products.

Two of these guidelines (one relating to products for human use, one to products for veterinary use) are devoted entirely to processed bovine serum. Serum producers ensure that processed bovine serum that is intended for use in the manufacture of pharmaceuticals meets the requirements established in these documents.

There is another guideline that has the force of law within the EU and in some other countries also. It applies to a wide range of ruminant-derived materials, not just bovine serum, and relates to the measures that must be in place to minimise risk due to TSE agents (see also: *“Is serum safe?”*). All processed bovine serum that is sold or used for manufacture of pharmaceuticals in or for Europe must be shown to comply. In order to establish compliance, companies must submit, for expert review, detailed product-specific information covering every aspect of sourcing, collection and processing.

Whatever the purpose behind the regulations, the ISIA and its members liaise closely with authorities to make sure that member companies are compliant. One of the key roles of the ISIA is to provide a recognised and authoritative link between its members and regulatory bodies and the scientific experts that are involved in the development and application of the legislation.

## ***Are there alternatives to bovine serum? What are the pros and cons?***

This is a question that is most often posed in the context of the culture media that are used in the manufacture of medicinal products.

The expression “serum-free medium” is widely used, especially when the pros and cons of different types of media are debated. A serum-free medium is often taken to be the same thing as a “chemically-defined” medium, but this is frequently not the case. Many serum-free media contain other animal-derived materials. Each one would have to be carefully investigated for safety and utility in the same way as has been done for bovine serum.

"Chemically defined" media may contain no animal-derived materials at all. They are in use, but not all cell types can be adapted to grow, or to grow adequately and normally, using such media. When they can be adapted to do so, then using totally chemically defined media may offer advantages. Using only “chemicals” means that a medium can be prepared with absolute precision, so once a particular “formula” has been shown to work well for a particular cell type, it can be reproduced exactly, time after time, and should always perform in the same way. Using a medium containing only chemical components may also help to simplify the manufacturing process. But it may take a lot of development work to arrive (or not) at that formula, and it is likely that such development will have to be done on a product-specific basis. Furthermore, the best yield obtained from chemically defined media systems may still be inferior to that obtained with serum-supplemented media.

There are many different types of cells that are grown, for many different purposes, in cell culture. Most cell types grow well in media that contain bovine serum and vigorous cell growth and division may be a critical characteristic for some applications. Some cell types have, so far, resisted all attempts to get them to grow and divide satisfactorily in media that do not contain serum.

Serum, like all biological materials, is subject to a degree of variability in its composition. This cannot be avoided. The higher the quality of the material, the less will be the variability, but it will never be eliminated. There are potential advantages – some batches of serum may be found to perform particularly well in certain applications. But there are obvious potential downsides too. Not all batches may perform in the way that a particular process requires. However, this inherent variability and its consequences are recognised by both processors and customers. They will conduct the investigations necessary to ensure that each batch that is supplied will perform according to expectations.

The fact that often only serum will provide what is required is recognised and accepted by both scientists and regulatory bodies. And, reassuringly, the safety in use of bovine serum has been established over many years. This is why high quality processed bovine serum is, and will continue to be, such a vital commodity.