

Advancing cell culture



There is a rich history of serum production in Logan, Utah, USA. In 1967, Dr. Rex S. Spendlove and fellow scientists at Utah State University (USU) were concerned about the generally poor quality of commercially available fetal bovine serum (FBS). This situation motivated them to begin collection and processing FBS for use in the university's virology research programs. In doing this, they developed methods which produced a high-quality serum unlike any commercially available. This on-campus serum project soon grew large enough to market the serum to wholesale buyers anxious to purchase serum of high quality. Demand for this high-quality FBS increased rapidly and profits from the sales supported the university's graduate research program in virology.

By 1975, the demand for the serum became so great that its production was produced off-campus to accommodate the increased demand. Thus, HyClone Laboratories, Inc., (originally Sterile Systems, Inc.) was formed by scientists who cared about and understood the needs of their fellow scientists.

Since that time, HyClone Laboratories has worked to improve serum quality through collection, processing, and filtration innovation. For example, serum endotoxin and hemoglobin are indicators of the care exercised during blood collection and processing. Through the use of a disposable closed-system cardiac puncture collection system and rapid processing, hemoglobin and endotoxin levels were significantly reduced. Additional innovations included serum filtration through three 0.1 µm pore size filters, minimizing the toxic effects of photo oxidation, providing more than 200 biochemical component assay results for each lot of serum, and consecutively, numbering each bottle during serum filtrations, producing large serum lots and assuring that each serum lot is not adulterated or diluted.

1967

Tissue Culture Laboratory established at USU. Fetal bovine blood collected and processed by advisor and students for use in virology laboratory. Improved procedures for producing FBS were developed.

1970-1975

FBS produced by students and sold wholesale through USU Foundation. Profits used to support graduate research in virology.

1975

FBS project taken off campus. Sterile Systems, Inc. (later HyClone Laboratories, Inc.) was incorporated. FBS was sold wholesale.

1977

10 years of R&D on blood collection and processing was applied in producing first lot of FBS for retail sale.

1978

Began assaying for endotoxin concentrations, to assure customers of low endotoxin contamination. Started serum component quantifications. Introduction of HyClone™ bovine calf serum.

1979

Consecutive numbering of serum bottles during filtration. Introduction of HyClone equine serum.

1980

Single 0.1 µm pore size filter added to filtration system. Introduction of HyClone porcine serum.

1981

Construction of new facility. Filtered serum through three 0.1 µm pore size filters.

1982

Began marketing antibodies, antisera, and Costar plastic labware. Sera produced under yellow lights and packaged in yellow bags to prevent phototoxic oxidation.

1983

Introduction of HyClone Sheep Erythropoietin, Nerve Growth Factor, and Characterized FBS.

1984

Produced HyClone Murine Erythropoietin and began marketing the Taggart hybridoma technology (THT).

1985

Remodeling and completion of facility expansion. Completion of new mass cell culture and radioisotope laboratories. Expansion of freezer, warehouse, and office space.

1988

Important innovative breakthrough in FBS processing with the new 40 nm filtration system.

1990

Dialyzed serum introduced to complement high-quality serum portfolio.

The advancements made while producing a high-quality sera have had long-lasting impacts with all serum producers today.

Definitions

Whole fresh blood is not modified, treated or processed and contains no additives. Whole blood may contain anticoagulants, but otherwise is not modified, treated or processed and contains no other additives.

Plasma is the liquid fraction of unclotted blood. After the addition of an anticoagulant to fresh whole blood, plasma is prepared by centrifugation of the mixture until the red and white blood cells separate from the liquid phase. The plasma is removed and may be stored frozen pending further use or processing.

Serum is the liquid fraction of clotted blood. It is depleted of cells, fibrin, and clotting factors. Serum differs from plasma in that anticoagulant is not added to the blood after collection from the animal. Serum is prepared by centrifugation until the clot and remaining blood cells are separated from the liquid phase. The serum is removed and stored frozen pending further processing (1).

About serum products

Serum origin

Serum origin refers to the country in which the raw blood was collected, and is not to be confused with the country in which the raw serum was sterile filtered. With HyClone sera, the country of origin is not a secret. The documentation you receive with your serum product will clearly state the country or countries of origin. This information is on the certificate of analysis and the certificate of origin. Countries of origin include:

USDA-tested

Intended for low-risk applications, HyClone USDA-Tested FBS is filtered in the U.S. and sourced from countries whose serum can be imported into the U.S. Areas include North America, Central America, Australia, and New Zealand. This serum is safety tested by the United States Department of Agriculture (USDA) when required, and considered to be foot-and-mouth disease (FMD)-free. As with all HyClone products, the certificate of analysis includes the specific countries of origin used in each lot.

South America

HyClone FBS, South American Origin is typically sourced from Brazil, Columbia, and Uruguay. South American FBS complies with EU regulations and meets the requirements of most Asian countries. South American FBS is available only to European and Asian customers.

United States

In-country processing of our US origin serum products is optimized for supply integrity, minimizing cross-contamination risks with other serum origins and types. Our US origin serum products include fetal bovine and bovine calf sera, as well as a variety of engineered or alternative species sera. Non-US origin products are manufactured in a separate facility, utilizing single-use technology to minimize risk of cross-contamination with other serum products.

Canadian Origin

Characterized fetal bovine serum sourced in Canada provides important proteins, hormones, growth factors, metabolites, and nutrients essential for cell culture. Canadian-sourced FBS is processed in the US.

New Zealand

The great expanse of the Pacific Ocean has protected New Zealand's islands from many outside influences, both geographically and biologically. One of the benefits of this isolation is that New Zealand has the fewest reported bovine diseases in the world, making it an excellent source of bovine serum. HyClone New Zealand origin characterized FBS and calf sera are carefully collected, processed, and filtered in our in-country facility.

Australia

HyClone Characterized FBS, Australian Origin is sourced from Australian abattoirs approved by USDA for export and inspected by the Australian Department of Agriculture (DA). We have found that Australian methods of animal husbandry exhibit excellent animal nutrition and healthcare. As with New Zealand, Australia is an isolated continent, thus making animal disease control and management easier than in most areas of the world (2).

Importance of origin

USDA considers certain countries to have negligible risk of bovine spongiform encephalopathy (BSE) and FMD. Some of these countries are New Zealand, Australia, and all countries in Central America where our sera are collected. However, the USDA permits only serum of New Zealand and Canadian origin to be imported into the U.S. without safety testing. Serum of Australian, Mexican, and Central American origin must be quarantined, sampled, and tested by the USDA for the presence of exotic strains of bluetongue virus and, in the case of Australian serum, Akabane virus as well. Once the quarantined serum has successfully completed safety testing, it can be sold and used in the U.S. without restriction.

The results of the safety testing are available to you with the purchase of a HyClone serum. There are many areas of the world which are prohibited from exporting serum into the U.S. You can be certain that our serum conforms to USDA regulations. Verification of origin is even more critical for serum users who are producing products requiring regulatory approval through the U.S. Food and Drug Administration (FDA), the USDA, or other regulatory agencies.

Traceability

We provide a verifiable document trail from the serum you receive to the geographic origin of the raw blood used in its production. It is critical to the safe use of serum. Issues related to BSE and FMD, for example, can only be controlled by use of serum from locations where FMD does not exist, or where BSE does not exist or has been detected at a very low level. We are dedicated to proving complete traceability on all serum products.

Method of collection

Controlling the initial collection of bovine blood is at least as important to serum quality as sterile filtration and aseptic packaging. A closed-system collection method (thoracic or venipuncture) and rapid processing are essential to a quality finished serum product. Low levels of endotoxin and hemoglobin are an excellent measure of the care with which collection and processing were done.

Some blood collectors use blood collection vessels, which are open to the environment of the packing house. This is especially true for calf blood collection where little care might be taken to avoid contaminants including stomach contents. In addition, collection of fetal blood by expressing it from the umbilicus into open containers is still practiced elsewhere. We do not use these practices in blood collection.

Collectors

Trained subcontracted collectors are very important to our quality serum products. As with any important procedure, skill and training are as essential in harvesting blood as in any step of its product preparation.

Age at time of collection

Blood sourced from an older animal will have different characteristics than blood sourced from a younger animal. Also, the type of feed will make a difference (i.e., old formula-fed veal animals vs range-fed animals). Some differences will be seen when comparing IgG, total protein, and albumin levels to name a few.

Lot size

Serum is a natural product resulting in lot-to-lot variability. Large lot sizes tend to minimize this variability. Additionally, if you utilize large quantities of serum, the cost per liter of lot quality control (QC) testing prior to purchase is reduced with large lots. Even if you use only small quantities, a serum supplier whose lot sizes are large (2000 to 3000 L) will provide the best opportunity of providing consistency order after order.

Filtration method

Common final filters used in serum processing have pore-size ratings ranging from 0.1 μm to 4.5 μm and may be used as a single filter or in a series. We recommend that your specifications require use of a minimum of 100 nm pore size-rated filters and that three filters in a series are used. This recommendation has become an industry standard. Forty (40) nm pore size-rated filters are the most retentive filters used to process commercial-sized lots. This method is used to produce our Defined Fetal Bovine Serum.

True pool technology

Processing using true pool technology means that the serum is pooled after filtration and before final packaging. If serum is pooled, filtered, and bottled directly after filtration, the serum will likely have increased variability from bottle to bottle. This variability is due to changes in filtration properties as hundreds of liters pass through the filters. With true pool processing, customers are ensured of more consistent quality from the first bottle filled to the last. Variability in serum components is natural and is the result of a variety of factors (age of the animal, origin, season collected, etc.). Large lots processed using true pool technology increase consistency and minimize variability within a lot.

Post-filtration treatments

The most commonly used post-filtration treatment is exposure to gamma irradiation. This is a powerful tool to further reduce the risk of adventitious viruses present in serum. The irradiation process used should be validated to deliver a minimum dose of 25 kGy. If you are manufacturing products for use in humans or in animals, irradiation should be a routine requirement.

Heat inactivation by exposing the serum to a temperature of 56°C for 30 min is also a common post-filtration process. Both services are available upon request.

Commitment to quality

All GE Healthcare's HyClone products are produced according to our quality system, which is certified to ISO 9001 and ISO 14385 guidelines.

Basic cell culture

Cell culture is defined as cultivation of individual cells outside the environment of the complete organism. The term *in vitro* is used to describe cell culture, meaning cells cultivated outside their normal environment. The term *in vivo* refers to cell growth within the source organism. You will also encounter the term tissue culture that is often used synonymously with cell culture. In addition to this synonym, tissue culture sometimes refers to *in vitro* cultivation of aggregates of cells from portions of organs. To prepare cells for cell culture, the organ or section thereof is diced and placed in an enzyme solution that dissolves the matrix binding individual cells together. Once the cells have been removed from the organ and enzymatically digested, they are isolated and placed into culture dishes or flasks. These cells thus placed *in vitro* are fed with a nutrient medium usually made by supplementing medium with animal serum. The basal medium (i.e., without serum) is composed of a balanced salt solution, vitamins, growth factors, carbohydrates, and other factors that provide the proper environment for cultivation of cells. There are many types of media currently on the market. The serum that supplements the medium can be from one of a variety of different species (bovine, ovine, equine, etc.). The one most commonly used is from the bovine species. Of the bovine sera that are used in cell culture, serum derived from fetuses (FBS) is most common. Other bovine sera are calf serum, iron-supplemented calf serum, and newborn calf serum. Other animal origin sera from pigs, horses, goats, or other small animals are sometimes used.

Initial culture systems are placed into an incubator where temperature is controlled. When cultures are set up starting with an organ, the resulting cell culture system is referred to as a primary culture. After several serial passages, the cells that stabilize in culture are referred to as established cell lines. A large variety of established lines are available.

Most cultured cells are the adherent type, that is, they adhere to the substrate (glass, plastic, or a treated surface). In order to pass or transfer cells, extra cellular matrix holding cells to the substrate (or each other) must first be dissolved. Trypsin is most often used for this purpose. In practice, the researcher will wash the cells free of the culture medium (proteins in the medium and serum will interfere with or neutralize trypsin), place an isotonic trypsin solution on the cell layer and carefully monitor for release of cells from the substrate. The trypsin is neutralized and the free-floating cells are recovered. These recovered cells will be passed into new flasks or dishes for further subcultivation; cell numbers are used by serial subcultivation.

There are a great variety of cells from many species used in cell and tissue culture. Research or production dictates the type of cells used with the *in vitro* system. For example, Chinese hamster ovary (CHO) cells, often genetically transformed to prepare desired end products, are widely used in the biotechnology industry. Other cell types are used in preparing antibodies or any number of products. In addition, many cells are used to study biochemistry, physiology, genetics, and more.

Uses and functions of serum

Cells will only survive, grow, and multiply *in vitro* if they are given nutrients and have an appropriate and protective environment. The cells are placed in a nutrient mixture known as the cell culture medium, which feeds and protects them. As cells have different requirements, different media are used. Along with medium, serum is added to the mixture and is essential for cells to grow adequately and normally. Serum acquired from bovine animals is primarily used as it is available in large volumes and is of high quality. High-quality medium and serum are necessary to support cell growth.

Uses

Serum is extensively used in such diverse areas as cancer research, production of monoclonal antibodies, development of therapeutics, preparation of vaccines, and diagnostic reagents (3).

Functions

Serum supplies micro- and macronutrients required by cultured cells. Micronutrients are essential elements needed by life in small quantities and include microminerals and vitamins. Microminerals or trace elements include at least iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, and molybdenum. Macronutrients are substances that provide calories or energy and are required in large amounts for growth, metabolism, and for other body functions. There are three broad classes of macronutrients: proteins, carbohydrates, and fats. Macronutrients also supplies protein such as fibronectin, which enhances cell attachment; and transferrin, which carries iron to cells. Serum also works as a carrier of minerals, fatty acids, and hormones, while naturally providing growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF). Serum also supplies metabolites, nutrients, amino acids, glucose, and vitamins (4).

Source of

- Hormones (testosterone and estrogen): cell growth and regulation
- Corticosterones: regulates the water and electrolyte balance
- Insulin: regulates carbohydrate and fat metabolism
- Growth factors: cell division
- Attachment/spreading factors (e.g., fibronectin)
- Trace nutrients: inorganic ions, lipids
- Carrier proteins (e.g., transferrin)

Role in

- Buffering (albumin)
- Redox (glutathione)
- Protease inhibitor (e.g., of trypsin)
- Viscosity (e.g., albumin) (5)

Industry economics

The supply of FBS is driven by fluctuations in the beef industry, as availability is not a result of FBS needs in the marketplace. These fluctuations impact all serum producers the same.

The economics involving FBS provide a unique opportunity, as few industries parallel the dynamics encountered in the serum business. Supply-versus-demand models for FBS do not follow typical economic principles. A traditional supply-versus-demand scenario demonstrates that supply usually meets the demand as factors are interdependent. In a FBS scenario, supply and demand operate independently of each other.

A typical FBS supply cycles from low to high during an 8 to 10 year span. FBS suppliers have little control over the supply as fetal bovine serum is a byproduct of the beef packing industry. The supply is dependent of various factors such as beef and dairy product consumption and feed prices; and environmental factors such as drought and floods, cattle import and export, and government farm policies. Attempts to rebuild a cattle herd causes ranchers to retain pregnant cows otherwise destined to be sold to beef packers.

While events such as droughts, floods, and disease outbreaks have relatively short-term effects on the supply, several factors will affect the long-term outlook. Worldwide beef consumption continues to decline as people look to alternative sources of protein, such as poultry. As consumption declines, fewer cattle are processed and less FBS is available. Along with the improved efficiency of beef production, advances in genetics, and better nutrition, there has been increased meat production with fewer animals. This results in a slow but steady decline in FBS availability.

Supply factors

- Periods of over/under supply
- Price increases/decreases
- Industry incentives

Impact on raw material supply

- Global meat consumption
- Weather
- Feed costs
- Government regulations

Factors that Influence FBS components

FBS components normally found in different production lots can vary in concentration for a number of reasons. These influencing factors can either occur at the abattoir, as the serum is in the raw form, or during the time, as a finished product.

Influencing factors at the abattoir

Inadequate handling of the blood can lead to the rupture of red and white blood cells with the release of organic and inorganic cellular compounds; blood cell metabolism when the blood is not processed soon after collection; and microbial contamination.

- **Diet** influences the composition of the blood. The diet of cows depends on the season of the year and on their geographical location. For example, in the northern sections of the country, cows graze in the summer and are fed hay in the winter. Feeds used in different geographical locations range from potatoes to cotton seed meal or citrus rinds. Soil conditions can also influence serum composition. For example, where selenium is present in low concentrations in the soil, the plants will contain relatively little selenium. As a result, the fetuses of cows feeding on these plants will have low selenium levels in their blood.
- **Undesirable contaminants** in cattle feed can enter fetal blood as a result of the dams (mothers) ingesting materials such as teratogenic alkaloids in poisonous plants. Feed and water can be contaminated with herbicides and pesticides. Water, hay, and pastures near highways or smelters can become contaminated with lead and other toxic heavy metals. Mold spoilage of cotton seed has been reported to cause aflatoxin contamination of milk. Therefore, aflatoxin contamination of FBS can be of concern in some situations.
- **Season of the year** when cows are generally bred to calve is in late winter or early spring. Therefore, most fetal bovine serum acquired during the winter months

are from animals in their third trimester. After the normal calving season, many of the fetuses are small. Since the concentrations of serum components change substantially with stage of gestation and the average fetal size differs with seasons of the year, it is not surprising to find that different lots of serum vary greatly in their concentrations of serum components.

- **Animal welfare** practices impact the quality of the serum. Factors such as emotional stress, pain, or injury experienced during time at the abattoir can stimulate the release of substances such as catecholamines that prepare an animal for flight or fight. GE encourages all abattoirs to practice the most modern and least stressful animal husbandry techniques for both the wellbeing of the animal and the quality of the serum.

Influencing factors of raw or finished serum

Serum that is in the raw state or finished product can be effected by auto-oxidation, photo oxidation, and holding of serum for extended periods in liquid or frozen state. There can also be inaccurate assay results obtained when high levels of certain components such as hemoglobin are present.

Size of production lot should be inversely related to the serum component variations. Larger lot sizes reduce the variations of component concentrations.

Bovine fetal blood availability

A bovine fetal blood availability chart is shown in Figure 1.

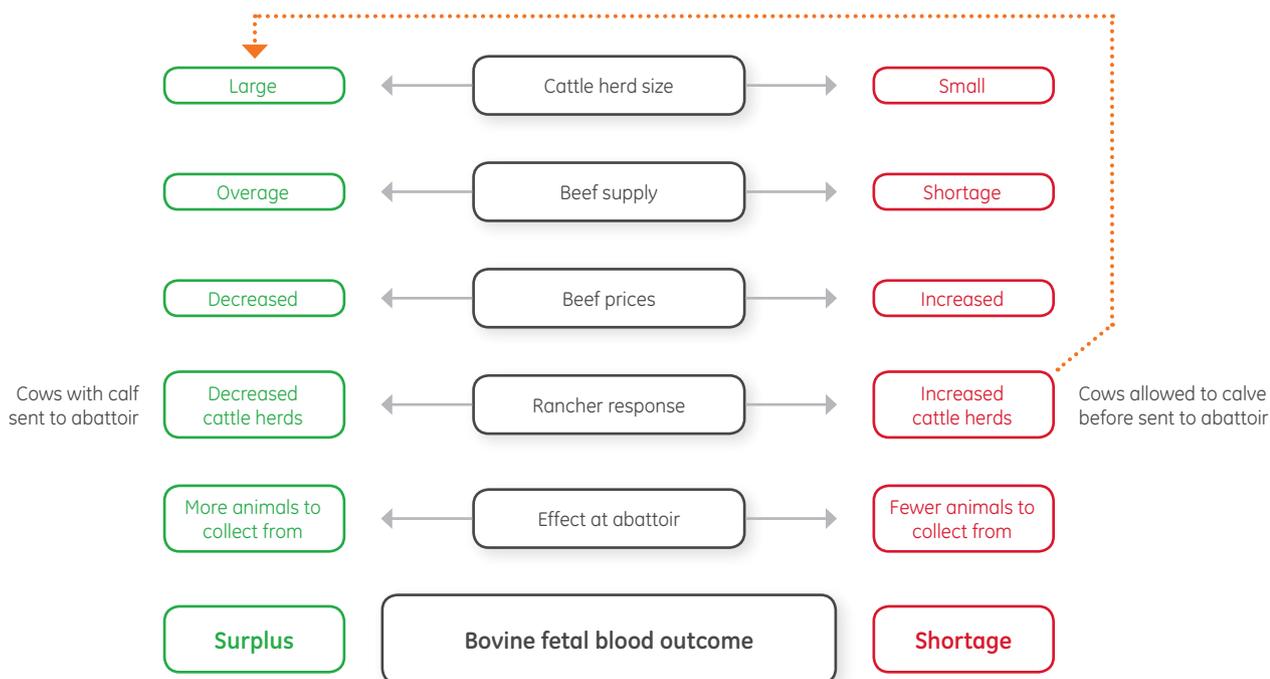


Fig 1. Bovine fetal blood availability.

FBS product flow, from field to finished product

The product flow chart for serum collection and processing in Figure 2 is specifically for U.S. FBS. Similar processes are followed for non-U.S. origin FBS produced at other locations.

The product flow chart for serum filtration and testing in Figure 3 is specifically for U.S. FBS. Similar processes are followed for non-U.S. FBS produced at other locations.

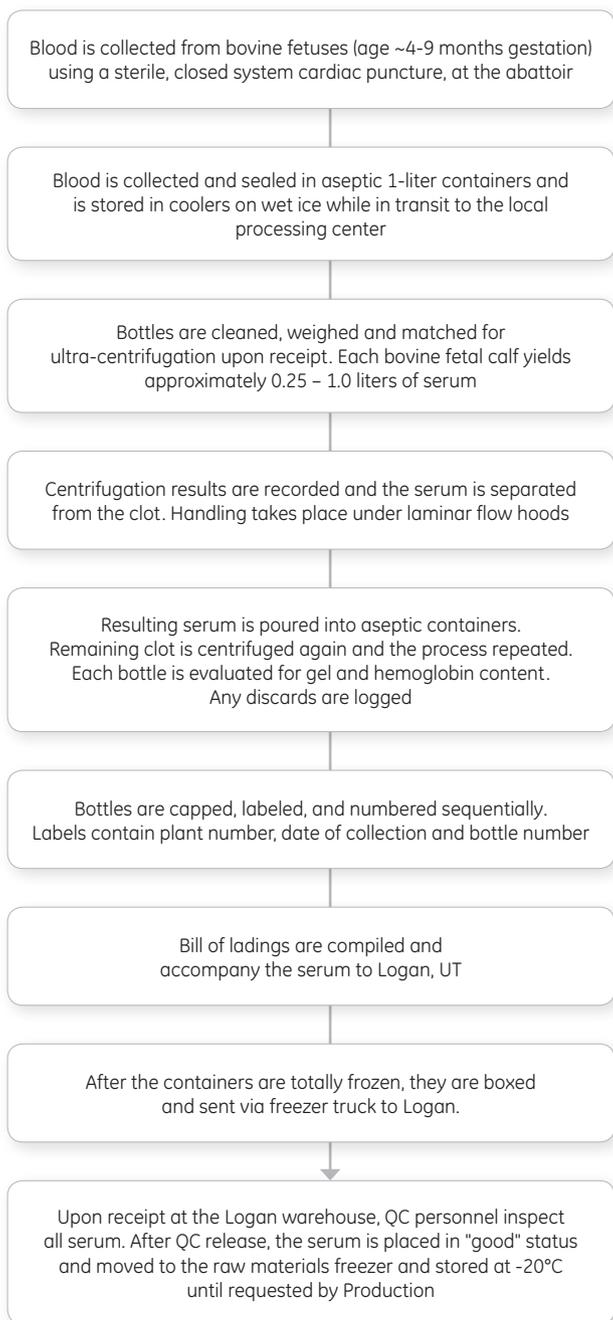


Fig 2. Collection and processing product flow.

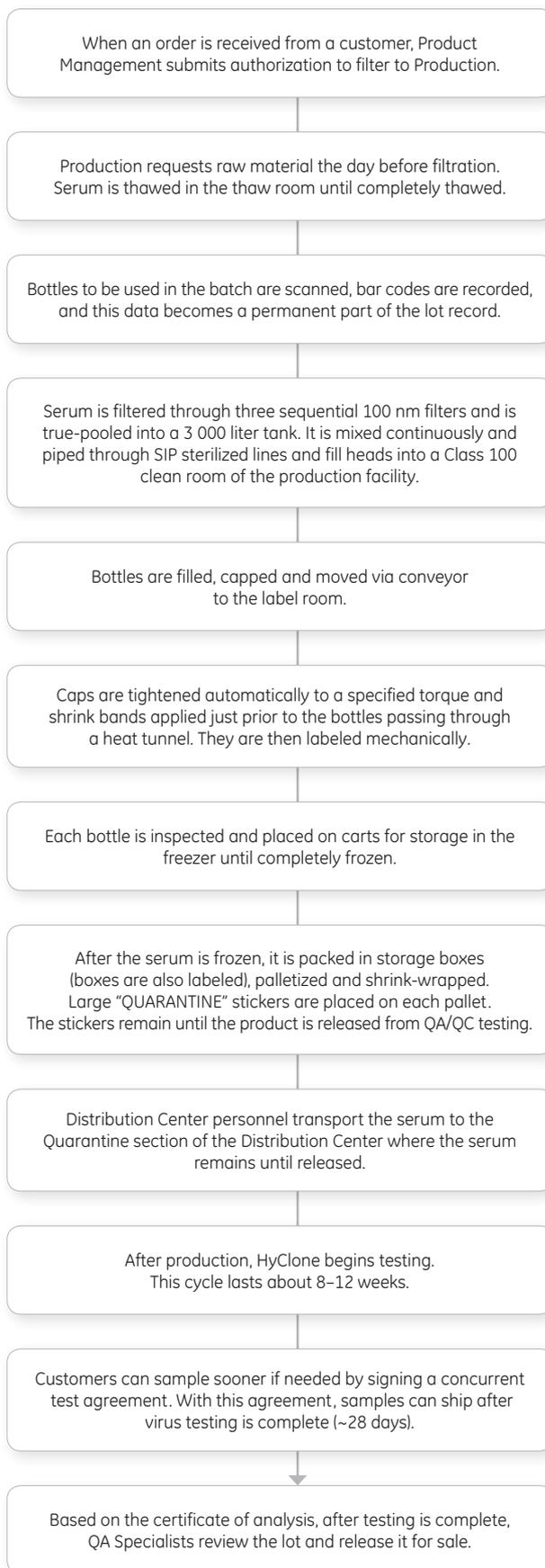


Fig 3. Filtration and testing product flow.



Global serum market outlook

The supply of FBS, a byproduct of the beef industry, is impacted by complex market conditions. For these reasons, GE Healthcare ensures adequate supply for our customers by not only sourcing FBS from several regions, but also working with a broad base of serum and raw blood suppliers. To ensure traceability and security of supply, raw serum is obtained in New Zealand, Australia, the United States, Canada, Central America, and South America.

References

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