INTRODUCTION

Serum is commonly used as a supplement to basal growth medium in cell culture. The most common type of serum used for cell growth is foetal bovine serum (FBS), also known as foetal calf serum (FCS). Foetal bovine serum is obtained from foetuses harvested in abattoirs from healthy dams fit for human consumption. Occasionally, there may be use of other bovine sera, such as newborn calf serum or donor bovine serum. In cell culture, serum provides a wide variety of macromolecular proteins, low molecular weight nutrients, carrier proteins for water – insoluble components, and other compounds necessary for in vitro growth of cells, such as hormones and attachment factors. Serum also adds buffering capacity to the medium and binds or neutralizes toxic components. Attempts to replace serum entirely with serum-free medium have met only with limited success.
The selection of a serum supplement for cell culture applications is primarily dependent on the chemical definition of the basal medium, the type of cell to be grown, and the culture system being employed.

COLLECTION

In the FBS manufacturing process, whole blood is collected aseptically in disposable sterile plastic bags and allowed to clot. Once the serum has been separated from the clot, it is pooled and frozen. Controlling the initial collection of foetal blood is a crucial factor in the quality of the final serum product. Only raw material that meets our specifications is approved for production.

RAW MATERIALS

Two distinct grades of FBS are available on the world market: USDA-Grade FBS and European-Grade FBS.

USDA-Grade FBS is produced from raw materials originating only from countries certified to be free of both BSE (Bovine Spongiform Encephalopathy) and FMD (Foot and Mouth Disease). This product can be freely imported into any country, and is the product of choice in **all** countries for manufacturing purposes. Furthermore only the use of this product allows researchers to send their cells, or the products of their cells, to collaborators in other countries with strict import regulations. All FBS processed in the Biological Industries plant is USDA-Grade.

Cat. No.	***Product Name***
04-001-1	USDA-Grade FBS
04-002-1	USDA-Grade FBS, Qualified for Human Embryonic Stem Cells
04-121-1	USDA-Grade FBS, Heat Inactivated
04-011-1	USDA-Grade FBS, Dialyzed
04-201-1	USDA-Grade FBS, Charcoal-Stripped

PROCESSING

Selected batches of serum raw material are thawed, tested for endotoxins and hemoglobin content and only the accepted material is pooled. The pooled raw material is thoroughly blended under refrigerated conditions and membrane filtered for sterility according to a well validated filtration protocol. Biological Industries processes FBS through a sequence of pre-filters and membrane filters. The final filtration step includes the use of three 0.1 micron sterilizing grade membrane filters in series.
After filtration, the serum is dispensed into bottles by an aseptic filling process which has been validated to insure sterility of the final product. Serum products are produced in a class 100 environment. All airflow on the filling bench is filtered through HEPA filters. The room is maintained under positive pressure to its surroundings. The filling environment is routinely monitored for total particle counts, viable particle counts and pressure.
After filling, the final product is quickly frozen to –20^{o}C and held in quarantine until all quality control tests have been completed.

FBS QUALITY CONTROL

Each lot of FBS is tested to confirm that the serum meets the written specifications.
Final product release is done after reviewing all production and quality control records to determine compliance with all established, approved written procedures.

Physical and chemical tests:
PH
Osmolality
Total proteins
Albumin
IgG
Hemoglobin

Microbiological tests:
Sterility tests: Bacterial and fungal sterility tests according to the current USP
Mycoplasma contamination: according to the Code of Federal Regulations (CFR), title 9, part 113 (culture method)
Viral contaminants: according to the protocols described in CFR, title 9, part 113 for Bovine Viral Diarrea (BVD), Infectious Bovine Rhinotracheitis (IBR) and Parainfluenza type 3 (PI3).
Viral antibodies: FBS is screened to determine the titer of neutralizing antibodies to BVD, IBR and PI3.
Endotoxins: the test is performed using the standard Limulus Amebocyte Lysate (LAL) with the kinetic turbidimetric method.
Biological performance (cell growth):

The cell growth tests are designed to check the efficacy of the FBS in promoting cell growth. Cells used are fibroblasts (MRC-5 diploid normal cells), epithelial cells (Hep-2) and hybridoma cells. Each test is conducted using the tested serum and a validated control lot. Growth promotion using MRC-5 cells is evaluated through several subculture generations to observe any evidence of cytotoxicity and morphological changes of the cells.
Hep-2 cells (ATCC, CCL 23): growth curve
MRC-5 cells (ATCC, CCL 171): 3 passages test
Hybridoma cells: cloning efficiency test

STABILITY

FBS stability at –20°C temperature was evaluated with several cell types for long periods. The FBS did not lose its performance for 55 months (4.5 years) with all the cells tested. Storage of FBS at –20°C without defrosting will maintain the quality of the FBS at least until the expiration date stated on the label.

QUALITY ASSURANCE

The FBS production process is carried out under controlled conditions in a controlled environment. The steam-in-place (SIP) sterilization , filtration for sterility and filling are validated as required for key aseptic processes. A dossier (Device Master record) exist for serum with all relevant data concerning serum production. The production process from the raw material to the final product in storage, as well as the quality control tests and results, are documented and filed to ensure traceability and control of the process.
Biological Industries products are manufactured in compliance with the quality management standard ISO 9001:2000 and certification is available upon request.
In addition, the FBS production process conforms to the In Vitro Diagnostics Directive (IVDD 78/79/EC) of the European Parliament. Therefore, our FBS received the CE mark making it eligible for sale in the European Union for *in vitro* diagnostics.
A Bovine Spongiform Encephalopathy (BSE) certificate of suitability had been issued to Biological Industries by the European Directorate for the Quality of Medicines (EDQM) via the process of certification of suitability of monographs of the European Pharmacopoeia.
All documents and certifications are available upon request.

SPECIAL PROGRAMS AND FBS PRODUCTS
(Prepared from USDA-grade FBS)

Serum reservation
Serum, as a biological material, represents an undefined mixture of components in which composition varies from one lot to the other. Some cell types are sensitive to the variations in serum performance. Customers are encouraged to evaluate serum samples with their own culture system and cells while we reserve the quantities of the specific lots until customer testing is completed. In this way, the customer may choose the best serum for his own applications.

Gamma-irradiated FBS
Gamma irradiation is a process which involves exposing material to high energy gamma photons released by radioisotopes, such as cobalt 60. This energy is transferred from the photons to the products, and is responsible for organism inactivation by ionization of nucleic acid bonds. The irradiation of serum is intended to provide complete assurance of viral inactivation. An extensive validation study has been performed to validate the irradiation process using FBS spiked with several viruses . We have demonstrated that properties and cell culture performance of FBS are not altered by gamma irradiation exposure up to 3.5 MRad.

Dialyzed FBS
Most cells grown in culture require a serum component of the growth medium to maintain their proliferative capacity. While whole serum is permissible for routine purposes, studies involving nutritional parameters or incorporation of labeled material require that the constituent under study be removed from the serum. The most commonly used method for removal of these constituents is dialysis of whole serum. For dialysis by diafiltration, serum is circulated through a hollow-fiber by the concentration method. The filtrate however, is replaced by the addition of physiological saline to the serum.

Heat Inactivated FBS
Heat inactivation of serum is performed by raising the temperature of the serum to 56°C and maintaining that temperature for 30 minutes. Heat inactivation is the method of choice to destroy complement, and to ensure that the cells will not be lysed by antibody binding.

Embryonic stem (ES) cells tested FBS
Embryonic Stem (ES) Cells are pluripotent cells derived from the inner cell mass of the blastocyst. The stem cells can be maintained *in vitro* for extended periods without loss of their capacity to differentiate to all cell lieages when reimplanted back into a blastocyst. ES cells may differentiate *in vitro* to a variety of cell types including neuronal, muscle, endothelial and hemapotoietic progenitors. General culture conditions are well established and usual require ES cells to be grown on an inactive feeder cell layer or with basement membrane components (Matrigel, Fibronectin, Laminin). When growing ES cells, one of the most of the most important parameters is the maintenance of the cells in the undifferentiated state. Pre-screening of the is essential before using it for the culture of ES cells. Various lots of sera are screened for the growth of Human ES cells using MEF's as a feeder layer for four passages. The following parameters are measured for the screening:
- Human ES cells colony morpholy
- Plating efficiency
- Differentiation rate: analysis of Human ES cells, surface marker expressed on the undifferentiated cells membrane (FACS analysis).

The results are used as an indication of the quality of sera for the growth and maintenance of undifferentiated stem cells.

Charcoal-stripped FBS
Charcoal-stripped FBS is used to elucidate the effects of hormones in a variety of in vitro systems. Studies include steroid- receptor binding, steroid regulation of cellular receptors, hormone secretion of various tissues and the function of thyroid hormones. The production procedure includes the use of charcoal and dextran to remove the hormones from the FBS.

FOETAL BOVINE SERUM FAQS

- What is the difference between Foetal Bovine Serum (FBS) and Foetal Calf Serum (FCS)?

They are the same and describe exactly the same serum product.

- My FBS contains precipitates. What are they and what should I do?

The precipitates contain fibrin (clot forming protein) and lipoproteins. This is a normal characteristic and will not affect product performance. To remove the precipitates, centrifuge the serum or simply let it settle to the bottom of the bottle and transfer the serum carefully to another sterile bottle.

- My FBS arrive partially thawed, what should I do?

Let the serum defrost completely, swirl the serum bottle gently and then re-freeze the serum. The quality of the serum will not have been affected.

- How do I heat-inactivate serum?

Serum heat-inactivation is performed in a waterbath at 56ºC for 30 minutes. The water level should be higher than the level of the serum. Monitor the temperature in a reference bottle containing water at the same volume during the heat inactivation. You must swirl the bottles to mix the serum every 5 minutes during heat inactivation to insure uniform heating.
Use a calibrated thermometer only!

- I have a jellylike fraction on the bottom of the bottle. What is it?

As a result of improper heat inactivation of the serum (temperature above 56^{o}C, more than 30 minutes), or inactivation without mixing the serum, a protein denaturation caused the jellylike fraction on the bottom of the bottle.
Do not use this serum.

- How should I thaw FBS?

We recommend thawing the serum at 2-8^{o}C. However, if necessary, you may thaw the serum at room temperature. Swirl the bottles gently to mix the serum during the thawing process.

- Why is the color of the FBS not exactly the same as with my previous lot?

The color of FBS is brown to brown-red. It is dependent mainly on the hemoglobin concentration in the specific lot. The color does not affect the FBS performance.

- Is the FBS raw material free of BSE?

FBS sold by Biological Industries has always been produced from raw materials originating from countries that are free of BSE according to the OIE (Office International des Epizooties).

- How can I be certain regarding the country of origin of the raw material that was used to manufacture sterile FBS?

Ask Biological Industries for a copy of the original raw material veterinary documents.

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Quality Control Laboratory - Foetal Bovine Serum

Cat. No: 04-001-1
Lot. No: 215165
Expiry date: April 30, 2011

Production Data:
Country of origin of raw material: *Panama*

Certificate of Origin of Raw Material:
Certificate no: 96610
Date: April 3, 2007

EDQM Certificate of Suitability: *R1-CEP 2000-112 Rev00*
Certification of Manufacturing Facility: *ISO 13485:2003*
CE Mark: *Registered*
Final lot volume: **546 liters**
Date of manufacture: **April 17, 2007**
Country of manufacture: *Israel (R1-CEP 2000-112 Rev00)*

Physical and Chemical Analysis

	Specification	***Results***
Appearance	Amber liquid	Amber liquid
pH	7.0-8.0	7.51
Osmolality	270-345 mOsmol/Kg	313
Endotoxins	<25 EU/ml	1.3
Haemoglobin Content	<30 mg/100ml	24.3
Glucose	Value mg/dl	95
Billirubin	Value mg/dl	0.1
AST (SGOT)	Value u/l	49
ALT (SGPT)	Value u/l	9
LDH	Value u/l	1279
Cholesterol	Value mg/dl	34
Triglycerides	Value mg/dl	63
Creatinine	Value mg/dl	3.18
Sodium	Value mmoles per l	139
Potassium	Value mmoles per l	10
Calcium	Value mg/dl	15.7
Phosphorus	Value mg/dl	9.6
Transferrin	Value mg/dl	0
Iron	Value µl/dl	162

Protein Profile

	Specification	***Results***
Total Protein	3.0-4.7 g/100ml	3.81
Albumin	1.8-3.0 g/100ml	2.07
Total Globulin	<2.9 g/100ml	0.74
α-Globulin	Value %	368
β-Globulin	Value %	76
IgG	<500 mg/liter	150

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Sterility Testing

	Specification	*Results*
Aerobic Bacteria	Not detected	Not detected
Anaerobic Bacteria	Not detected	Not detected
Fungi	Not detected	Not detected
Mycoplasma	Not detected	Not detected

Virus Testing

	Specification	*Results*
Bovine Viral Diarrhea (BVD)	Test & record	Not detected
Parainfluenza Type 3 (PI-3)	Test & record	Not detected
Bovine Rhinotracheitis (IBR)	Test & record	Not detected
Blue Tongue Virus (BTV)	Test & record	Not detected
Bovine Spongiform-Encephalopathy (BSE) and Foot and Mouth Disease (FMD) have not been reported in the country of source of the raw material for this lot.		

Cell Culture Tests (Growth Promotion)

	Specification	*Results*
MRC-5 (3 passages)	>80%	pass
HEp-2	>80%	pass
Hybridoma	>80%	pass

Name: A.Herman
Position: Quality Control

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