

TECHNICAL POSSIBILITIES OF SERUM REDUCTION IN BIOLOGICAL PRODUCTION SYSTEMS

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EXECUTIVE SUMMARY

- Bovine serum use is still very significant (5-800 kL/year) and shows an increasing trend
- Serum is a multi-function supplement, not a single nutrient. Its role must be evaluated case by case.
- Combination of different strategies can lead to effective substitution of serum, depending on function of serum:
 - Gradual adaptation
 - Design of Experiment (DoE) optimization
 - Spent Media Analysis
 - Intracellular metabolomics workflow
 - Secretome analysis
 - Multi-omics(metabolomics, proteomics) modeling
- Several successes became industrial standards/well demonstrated e.g. CHO, HEK293(T), T cells/CAR-T, BHK-21, PSC-derived cells
- Advancements on several fields: MSCs, NK cells, MDCK
- Technical and cost challenges on simpler applications: e.g. Vero, CEF and primary cells

GLOBAL BOVINE SERUM USE

Public data are sparse; most quantitative estimates refer to fetal bovine serum (FBS), not all bovine sera.

500–800k L/yr

Estimated global annual FBS supply

Order of magnitude, not audited global litres

1–2M

Bovine fetuses/year implied by recent reviews

Interpretation: supply is driven by slaughterhouse recovery from pregnant cattle — a co-product market with weak incentives to expand on demand.

Why this matters

A factor-of-two spread in market reports means bottom-up serum consumption should be presented as ranges, not point estimates.

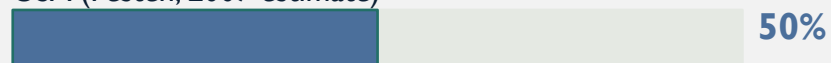
GEOGRAPHY: COLLECTION ORIGIN AND USAGE MARKETS

FBS collection is linked to cattle-processing systems; demand is concentrated in biopharma and cell-culture hubs.

Older supply-origin estimates

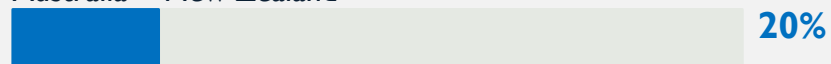
Share of annual FBS supply reported in reviews

USA (Festen, 2007 estimate)



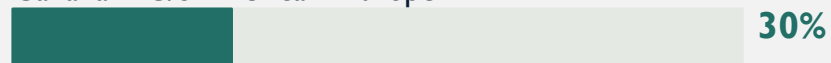
Later ISIA information cited by Weber: USA ≈26%.

Australia + New Zealand



Part of historic high-traceability supply base.

Canada + C/S America + Europe

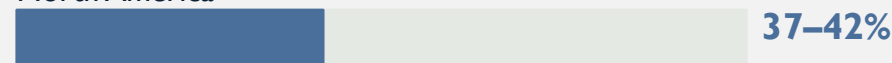


Remainder in 2007 estimate.

Demand/revenue geography

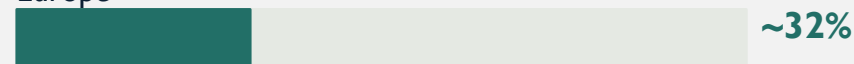
Market-revenue share, 2025: reported ranges

North America



Grand View: 37.41%; Mordor: 41.55%.

Europe



GVR Europe revenue \$727M vs \$2.3B global.

Asia Pacific + RoW



Asia Pacific reported as fastest-growing region.

Caution: origin estimates are old and partly conflicting; they are best used to illustrate vulnerability to cattle cycles and collection infrastructure, not precise 2026 shares.

Supply risk is global even when manufacturing demand is regional — alternatives reduce both process variability and geopolitical/raw-material exposure.

BOVINE SERUM IS A MULTI-FUNCTION SUPPLEMENT, NOT A SINGLE NUTRIENT

type fingerprints (relative, lot-dependent)

Core serum functions that must be replaced or controlled when moving to serum-free / xeno-free media.

Functional component classes found in bovine serum

Function / chemical class	Representative serum components	Why they matter in biomanufacturing media
Mitogenic & differentiation signals	IGF-I/II, FGF, EGF, PDGF, TGF-β, cytokines, insulin-like activity	Drive survival, proliferation, cell-state maintenance and productivity; may also shift viral susceptibility or differentiation.
Transport, buffering & protection	Albumin/BSA, transferrin, lipoproteins, fetuin, α1-antitrypsin, α2-macroglobulin	Carry lipids, trace metals and hormones; reduce oxidative/protease stress; stabilize labile factors and cells.
Attachment & matrix-like activity	Fibronectin, vitronectin, laminin, serum spreading factors, fetuin	Support adherent cell expansion, microcarrier processes and recovery after thawing/passaging.
Low-molecular nutrients	Amino acids, glucose/fructose, vitamins A/B/C/E, cholesterol, ethanolamine, fatty acids, Ca/Fe/Se/Zn	Fill gaps in basal media; support membrane synthesis, redox homeostasis, energy metabolism and biosynthesis.
Immunological / risk-bearing components	IgG/γ-globulins, complement, endotoxin, adventitious-agent risk; undefined batch-specific factors	Can inhibit cells or interfere with immunoassays/purification; major driver for defined, serum-free media in GMP settings.

APPLICATION-DRIVEN VIEW: WHICH SERUM FUNCTIONS MUST BE REPLACED?

The relevant “serum replacement” is not universal — it depends on the cell substrate, product and process mode.

Application	Typical cell substrates	Serum-derived functions that matter	Components or replacement levers	Manufacturing direction / comments
Viral vaccines	Vero, MDCK, BHK-21, MRC-5, CEF/primary cells	Attachment/recovery of adherent cells; early expansion ; protection from shear/proteases;	Albumin; transferrin/iron; insulin/IGF; lipids/cholesterol; fibronectin/vitronectin or microcarrier coatings; vitamins/trace elements; hydrolysates/peptones	Strong push toward serum-free/animal-component-free processes to reduce adventitious-agent risk; many vaccines still use legacy serum-containing seed or expansion steps. [7,10,13]
Veterinary biologics	BHK-21, Vero, MDBK, primary avian/bovine/porcine cells;	Robust growth of legacy substrates; attachment /microcarrier performance; viral amplification;	FBS/NBCS/other bovine sera in legacy processes; peptone/hydrolysate-based SFM; albumin/transferrin/lipid/attachment-factor modules; heat-inactivation where complement matters.	More heterogeneous than human biologics; serum-free veterinary vaccines have been demonstrated, but conversion is product- and cell-line-specific. [7,13]
mAbs & recombinant proteins	CHO, HEK293, PER.C6, hybridoma; increasingly suspension/high-density culture	Nutritional and protective functions rather than broad serum biology; media composition can impact product quality	Chemically defined basal media + feed; amino acids, glucose, vitamins, trace metals; lipids/ethanolamine/cholesterol; insulin/IGF alternatives; transferrin or Fe-citrate; anti-shear protectants.	Serum-free/chemically defined media are now the default for scalable recombinant-protein manufacturing. [9,10]
ATMPs / cell therapies	MSCs, T/NK cells, HSPCs, PSC-derived cells, fibroblasts/chondrocytes /keratinocytes	Expansion with phenotype preservation ; differentiation control ; xeno-antigen avoidance; matrix/attachment signals for adherent or stem-cell cultures.	Human platelet lysate or human AB serum; HSA; human/recombinant transferrin; recombinant cytokines/growth factors ; defined ECM/peptides; insulin/selenium/lipids; cell-type-specific cytokine cocktails .	Regulatory and biological rationale favors xeno-free and preferably defined serum-free systems, but hPL/human supplements remain common bridging strategies. [8,11,12]

18-27%

EXAMPLE ON MYCOPLASMA

- Mycoplasmas are simple
- Nutritional and carrier functions
- Protection vs. oxidative stress
- Still not all functions are known:

Complex media elements were used



Component	<i>M. pneumoniae</i>			<i>M. hyopneumoniae</i>
	vB10	vB13	vB13m	vH6
CMRL-1066	0.5 ×	0.5 ×		0.5 ×
L-Cysteine			100 µg/mL	
Glucose	7.5 g/L	7.5 g/L	7.5 g/L	1.5 g/L
Glycerol	0.025%	0.025%	0.025%	0.01%
Glutamine	2 mM	2 mM		2 mM
PPLO	15 mg/mL			
Yeastolate		10 mg/mL	10 mg/mL	7.5 mg/mL
RNA	1 mg/mL	1 mg/mL		
Spermine	10 µg/mL	10 µg/mL		10 µg/mL
Thioctic acid	0.2 µg/mL	0.2 µg/mL		0.2 µg/mL
Pyridoxamine	0.5 µg/mL	0.5 µg/mL		
Nicotinic acid	0.5 µg/mL	0.5 µg/mL		
Riboflavin	0.5 µg/mL	0.5 µg/mL		
Choline	0.5 µg/mL	0.5 µg/mL		
Cholesterol	33.3 µg/mL	30 µg/mL	30 µg/mL	30 µg/mL
Phosphatidylcholine	40 µg/mL	40 µg/mL	40 µg/mL	40 µg/mL
Sphingomyelin	40 µg/mL	40 µg/mL	40 µg/mL	10 µg/mL
Palmitic acid	16.6 µg/mL			
Oleic acid	20 µg/mL			
Nonlipidated BSA	0.33%			0.5%
2-Hydroxypropyl-β-cyclodextrin		5 mg/mL	5 mg/mL	
Sodium pyruvate				2.2 g/L
Thymidine				40 µg/mL
Adenosine				10 µg/mL
Cytidine				10 µg/mL
Guanosine				10 µg/mL
Thymine				10 µg/mL
L-α-Phosphatidyl-DL-glycerol				10 µg/mL
1,2-Dipalmitoyl-sn-glycero-3-phosphocholine				20 µg/mL
HEPES	50 mM	50 mM	50 mM	
Ampicillin	100 µg/mL	100 µg/mL	100 µg/mL	100 µg/mL
Phenol red (pH 7)	0.0035%	0.0035%	0.0035%	0.0035%
NaOH (to adjust pH to 7.7)				

STRATEGIES FOR SERUM REMOVAL

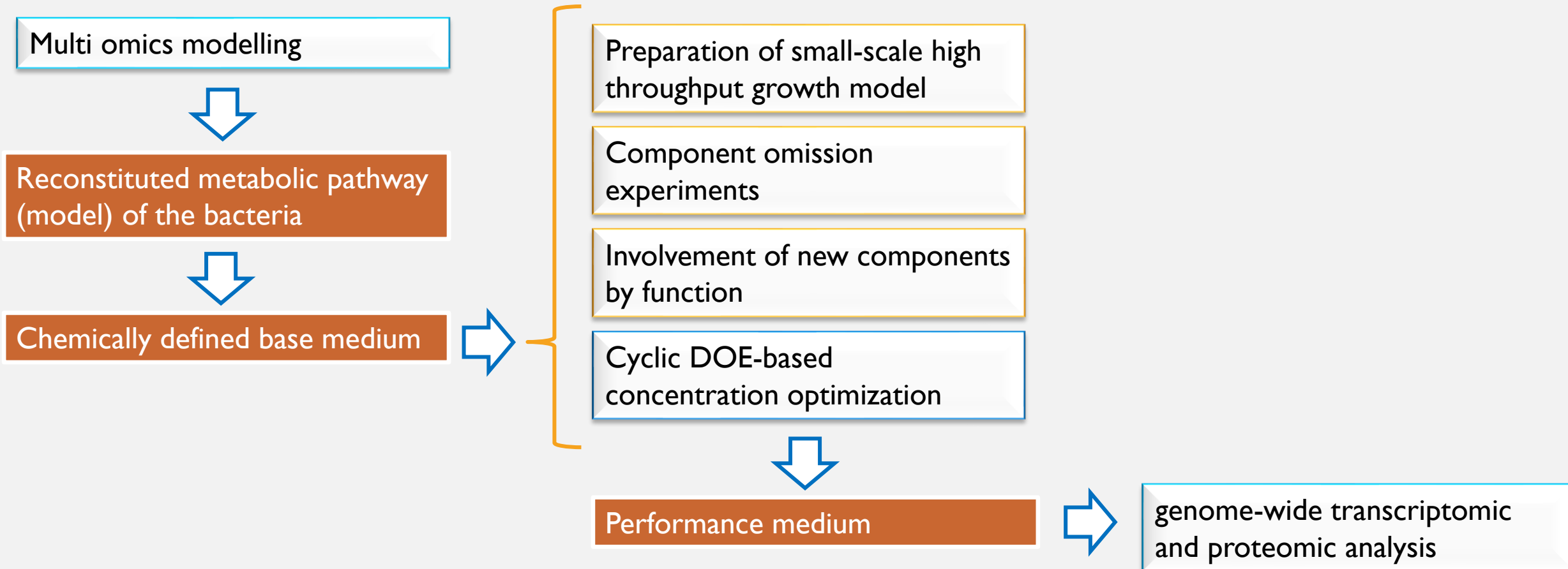
Strategy	Working Method	Applications/Limitations
Gradual adaptation	Serum is gradually decreased during cell passaging. May be combined with medium exchange to existing more complex medium.	Works for nutritional and protective functions and for limited cell types, may lead to only decrease of serum concentration. Expl. HEK293 ¹
DoE-based media optimization	Statistical approach using other complex medium components and/or recombinant proteins.	Works mostly for nutritional and protective functions and for limited cell types, may lead to only decrease of serum concentration. Expl. Vero ²
Spent media analysis	Fresh media composition is compared with time course of used medium (e.g. LC-MS), limiting components are added	Traditionally focuses on nutritional and protective functions, but can be extended.
Intracellular metabolomics workflow	Cell extract compositions are analysed (e.g. LC/GC-MS), metabolic pathway modelling and bottleneck identification	Focuses on nutritional function. Complex and sensitive, requires high level mathematical toolkit.
Secretome analysis	Identify endogenously produced growth factors and proteins, adds limiting factors	Phenotype preservation; differentiation control. Mostly for ATMPs
Multi-omics (metabolomics, proteomics) modeling	Integrate metabolomics, proteomics, transcriptomics and modeling.	In theory can cover all serum functions. In practice complete modeling is rather difficult.

+ feeding strategy (data are not static)

COMBINATION OF THE METHODS

The Mycoplasma Example

Min. 4 years + 3 M USD



ECONOMY OF SERUM REMOVAL

Cost Estimates

- A Spent media analysis + DoE with readily available complex and individual components at a major biotech supply company 0.8-1.4 M USD.
- Same approach without access to supply media components at high level scientific institute 5-800 kUSD.
- Additional costs: transfer, raw material validation, scale-up, regulatory and process validation batches, regulatory procedure + increased media costs
- + risk of dependency on single supplier

Min. 4 years + 3 M USD

Vet market:

- a dose of Mesenchymal Stem Cell product: 300-1200 USD
- a dose of poultry vaccine is ~3.5-10 cents

Gain Estimates

- Shorter delivery and release time of medium, lower inventory : 1 x gain ~ 500 kUSD
- More robust product titer > can be achieved by serum concentration increase or selection of serum
- Lower media test costs (but higher media cost)
- Lower risk of contamination
- More stable supply

In case of low price products serum removal may have economical barriers (COG)

BOVINE SERUM REPLACEMENT STATUS

ROUTINE / INDUSTRIAL

PROCESS-DEMONSTRATED

EMERGING / PARTIAL

Cell substrate / platform	Primary Use	Serum-free / xeno-free replacement status	References
CHO	mAbs, recombinant proteins	ROUTINE / INDUSTRIAL Chemically defined, serum-free fed-batch media are the default route for scalable mAb/protein production.	Rodrigues et al. 2012; Li et al. 2021
HEK293 / 293T	recombinant proteins, viral vectors	ROUTINE for transient expression and vector production Serum-free suspension HEK293 is widely used; transient transfection and productivity remain process-optimized.	Pham et al. 2005; Tan et al. 2021
MDCK	influenza vaccines	PROCESS-DEMONSTRATED to advanced Serum-free suspension/microcarrier MDCK has produced H1N1/H5N1 vaccine virus at high yield.	Hu et al. 2011; Huang et al. 2015
Vero	rabies, polio, JE, viral vaccines	PROCESS-DEMONSTRATED Adapted serum-free suspension Vero processes can support rabies and other viral vaccine production, but conversion remains product-specific.	Paillet et al. 2009; Rourou et al. 2019
BHK-21	FMD, rabies, veterinary vaccines	INDUSTRIAL Animal-component-free chemically defined suspension BHK-21 scaled to 200 L for FMD antigen production. Introduced at industrial level.	Paillet et al. 1995; Park et al. 2021
MRC-5 / CEF / primary cells	legacy viral vaccines	EMERGING / PARTIAL Serum-free MRC-5 media have reached comparable growth/titers in development studies; primary/CEF substrates remain more product-specific.	Hachmann et al. 2018; Koh et al. 2026

BOVINE SERUM REPLACEMENT STATUS

ROUTINE / INDUSTRIAL

PROCESS-DEMONSTRATED

EMERGING / PARTIAL

Cell substrate / ATMP	Serum role in legacy culture	Serum-free / xeno-free replacement status	References
MSCs	Expansion, attachment, trophic-factor support	PROCESS-DEMONSTRATED / widely used bridge Fully defined SFM/XF is source- and donor-dependent.	Rodrigues et al. 2012; Li et al. 2021
T cells / CAR-T	Activation, survival and expansion; cytokine synergy	ROUTINE to process-demonstrated Activation reagent, cytokines and bioreactor mode are key.	Pham et al. 2005; Tan et al. 2021
NK cells	Survival/activation during expansion	EMERGING / PARTIAL Bovine serum can be avoided, but feeder/cytokine dependency often remains.	Hu et al. 2011; Huang et al. 2015
HSPCs / CD34+ cells	Survival during manipulation; cytokine responsiveness	PROCESS-DEMONSTRATED Preserving primitive HSC phenotype is the limiting attribute.	Paillet et al. 2009; Rourou et al. 2019
PSC-derived cells	Historically serum/feeder support; differentiation control	ROUTINE for PSC expansion; product-specific for final cell type.	Paillet et al. 1995; Park et al. 2021
Keratinocytes / fibroblasts / chondrocytes	Attachment, proliferation, matrix support	MIXED Keratinocytes strongest; chondrocyte phenotype and fibroblast productivity need assay-specific validation.	Hachmann et al. 2018; Koh et al. 2026

TAKE HOME MESSAGE

In spite of increasing number of serum free applications, bovine serum use still does not decrease

Recombinant-protein platforms are the most mature; viral-vaccine substrates increasingly have serum-free suspension/microcarrier options, but Vero, BHK-21 and diploid/primary cells still require product-specific comparability work.

Economical interests are against new development in low price products

In cell therapies, “serum-free” does not automatically mean fully defined — human Platelet lysate/human serum remove bovine risk but introduce human-donor variability and need for blood-product controls.