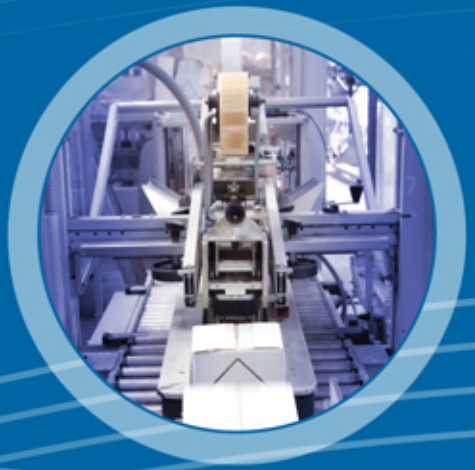
Connecting People, Science and Regulation

Serum and Safe Use
The Supplier's View



International Serum Industry Association

Rosemary J. Versteegen, Ph.D.

Disclaimer: Not a mycoplasmologist
But I love mycoplasma filtration!!!

Agenda

- What is ISIA?
- What are ADBPs?
- What are they used for and why?
- Serum processing
- Mycoplasma and serum
- Concerns and solutions
- What are the issues facing the industry in regard to Mycoplasma

ISIA

- International association of serum producers, suppliers and customers, founded in 2006
- Members supply more than 90% of all animal serum used globally
  - No human materials or food applications
- Members are also major suppliers of other animal derived products
  - Proteins, Antibodies, Enzymes, etc.
- Expanded mandate includes all animal derived products

Mission

- **ISIA** shall establish, promote and assure compliance with uncompromised standards of excellence and ethics in the business practices of the global animal serum and animal derived products supply industry.

- Primary focus will be on safety and safe use of serum and animal derived products through proper origin traceability, truth in labeling, and appropriate standardization and oversight.
- Work to educate stakeholders on the scientific foundation of the safe use of serum and animal derived products.

Members

- Abattoir Basics Company
- Access Biologicals
- APS
- ATCC
- Animal Technologies
- Atlanta Biologicals
- Axenia Biologics
- Bio Arra
- BioCell
- BioDux
- Biological Industries
- BioMin
- BioNord Sera
- BioSul
- BioWest
- Bovalco
- ByProductos
- Central Biomedia
- Enhancell Cell Technology
- Gemini Bio-Products
- Hemostat

- HyClone/GE Healthcare
- Life Technologies/ThermoFisher
- Lampire
- Lonza
- Millipore/Merck
- Mediatech
- Moregate Biotech
- Patricell
- Pelfreez
- Proliant
- River City
- Rocky Mountain Bio
- Selborne Biological Services
- Seradigm
- Sera Lab
- SerumTech
- Sigma-Aldrich
- TCS
- Vacca
- Wisent

Associate Members

- AmBio
- Becton Dickinson
- Idexx
- J&J
- LGC
- Merck and Co
- Oxoid
- Sheffield
- Viral Sciences

Critical ISIA Programs

- Quality and Standardization
  - USP collaboration and standard development
- Regulatory Involvement
  - Worldwide interactions
  - Education
    - Development
    - Implementation

ISIA Network

Traceability

- The ISIA Traceability Certification Program benefits members and customers
  - Regulatory agencies.
- Certification is performed by third party independent auditors using the Audit Guidelines laid down in the ISIA Traceability program
  - And it works!!
- Members use the ISIA Quality Mark as a symbol of their commitment
- ISIA keeps a current listing of those companies approved to use the ISIA Quality Mark on our website

The Traceability Quality Seal

Serum

The Serum Saga

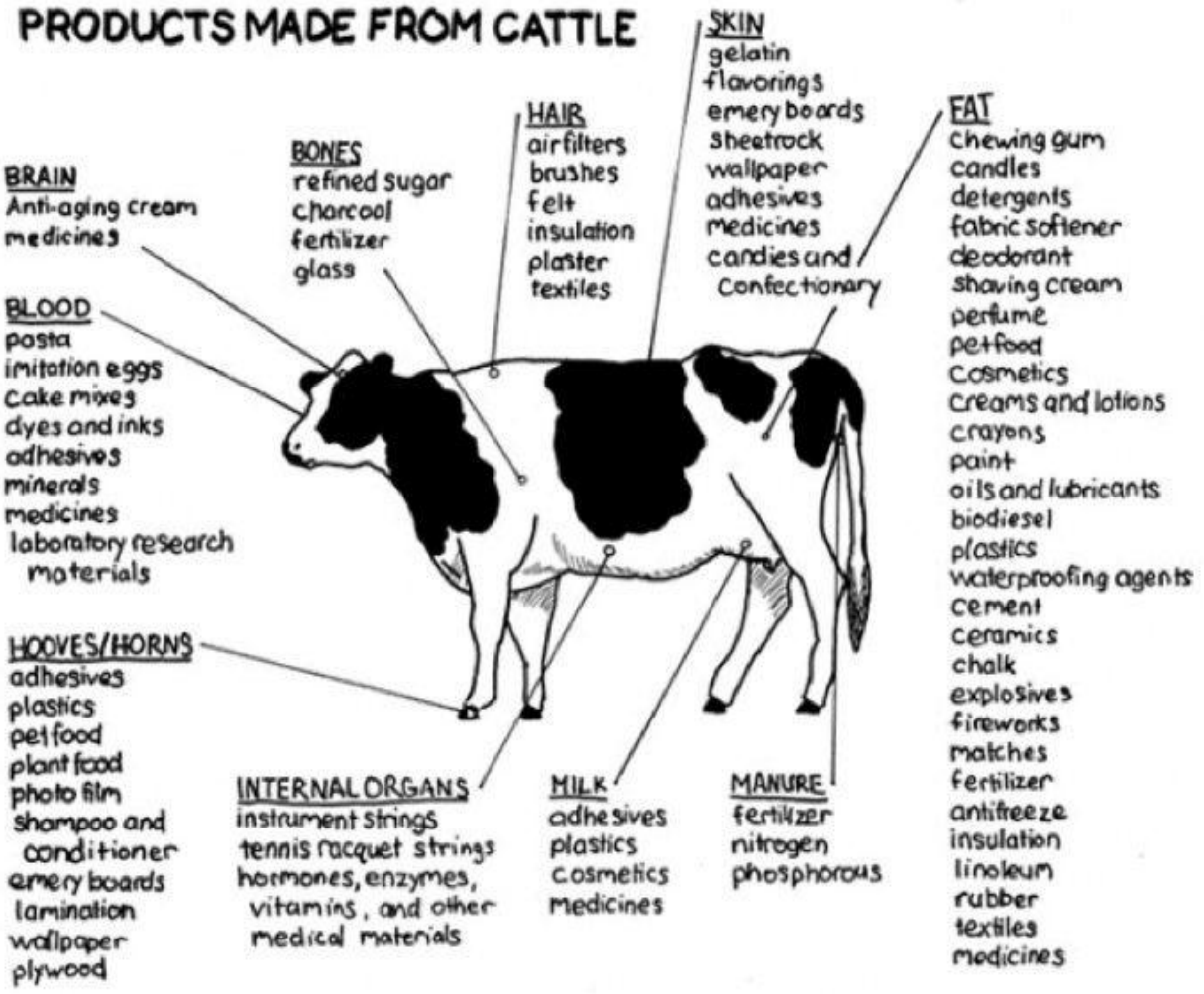
- Fetal bovine serum (FBS) has an impressive history - 65 years of safe use
- No known substance has the same broad spectrum cell growth promoting capabilities
- Over time concerns have arisen over FBS utilization in healthcare related manufacturing (adventitious agents)
- Considerable efforts have been made to remove animal derived materials and FBS from use with limited success
- Demand for serum continues to increase, suggesting that serum will be in use for a very long time
  - Can't live with it……..

End Users

- The Biotechnology industry is a multibillion dollar business affecting healthcare worldwide
  - Animal sera and other derived products are critical in this segment
- Animal sera and derived products are used in
  - Life science research
  - Production of human and animal vaccines
  - Production of biopharmaceuticals
  - Production of diagnostic products
  - Cell based systems for safety testing

For simplicity's sake we will focus only on serum now!

Use of Bovine Materials



The Serum Industry

- Total volume of Fetal Bovine serum (FBS) used annually exceeds 800,000 liters
  - Liters of other serum exceed 1,200,000
- More than $350 Million dollars of serum sold annually
  - 85% of that revenue is from FBS
- Pricing within FBS
  - NZ and Australian serum used in Biopharmaceutical Manufacturing sells for ~10 times more than South American serum used in research
- Pricing of other serum significantly lower than that for FBS

Regulatory Concerns

- Animal by-products not intended for human consumption are a potential source of risk through the presence of adventitious agents and chemicals
  - Foot-and-mouth disease
  - TSEs :bovine spongiform encephalopathy (BSE)
  - Dioxins
  - Other viruses and Mycoplasma
- Potential impacts from the above
  - public and animal health
  - safety of the food and feed chain
  - consumer confidence

Serum Selection

- Serum lots for purchase are selected based on customer application and performance

- Disease concerns are dependent on prevalence of disease and customer application
- The customer will evaluate multiple samples from various lots and manufacturers
- The supplier is almost never aware of the scientific information used to make the buying decision

Mycoplasma and Serum

Serum Manufacturing

- By-product of the meat industry
- Some cows come to slaughter pregnant
  - Current drought situation
- Fetuses collected only from dams deemed fit for human consumption by competent government authorities.
- Blood collected in a separate “clean” area
- Bags of blood placed on ice while blood is allowed to clot

- Bags centrifuged under refrigeration
- Serum harvested aseptically, filled into containers
- Sampled, bottled, labeled, snap frozen

Serum Manufacturing

- Transported frozen to final processing facility
- Thawed under temperature controlled conditions
  - pool of up to 2,500 liters
- Aseptically filtered using a validated process
  - filter train of pre-filters and three 0.1 micron final filters
- Aseptically filled, sealed, labeled, snap frozen and stored
- Quality control tested
- Further treatment frequently required

Quality Control Testing

- Serum is quality control tested for a broad range of parameters to the highest standard required
- Methods used for mycoplasma testing currently include:
  - Barille, MF and Kern, J (1971), P.S.E.M.B. 138, 432 (modified)
  - 9CFR 610.30
  - FDA PTC 1993
  - EP Chapter 2.6.7
  - USP Chapter <63>
  - Others

Mycoplasma Testing

- Methods are not harmonized worldwide
  - Volume
  - Time
  - Control organisms
  - Growth media
- Cell culture methods take 28 days and result interpretation can be subjective
- Old technology

Mycoplasma Testing

- Move towards nucleic acid based testing for mycoplasma using standardized methods (WHO IS standard)
- Several mycoplasma detection kits are commercially available

- Many companies currently use PCR testing to shorten times to obtain results internally
- ISIA explanatory white paper on PCR testing in process
- Standardization will benefit all!!

Mycoplasmas in cell culture

Mycoplasma in Cell culture

- More frequent in cells in continuous culture
  - Suggests infection comes from growth materials or human contamination

- ? large scale catastrophic contamination events known to be caused by bovine or porcine mycoplasmas

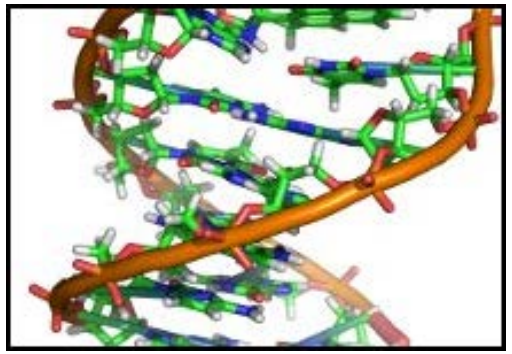
Gamma Irradiation

- Serum for use in pharmaceutical manufacturing is routinely gamma irradiated
- Viral reduction process is validated based on CPMP/BWP/268/95 and ICH Topic Q5
  - All suppliers have validated the irradiation process for the material and the facility used
  - Dossier available to customers
- Standard procedure is to irradiate with 25 to 45 Kgy
  - Serum is on dry ice throughout the process
  - Customers titrate the balance between biological activity impact and risk
  - Customers may request higher dosages

Gamma Irradiation

- Viral inactivation is well understood and shown in multiple studies to significantly reduce viral burden with the exception of small non-enveloped viruses

- Mycoplasma have been shown to be highly sensitive to gamma irradiation in multiple studies
  - 25 Kgy gives 6 log reduction



Read all about it!

Gamma irradiation of animal sera for inactivation of viruses and mollicutes – A review

Raymond W. Nims , Gay Gauvin, Mark Plavsic

Biologicals Volume 39, Issue 6, November 2011, Pages 370–377

Other Treatment Methods

- Heat
  - 56ºC for 30 minutes can be used for serum
  - Mycoplasma sensitive to 45ºC for 30 min or 60ºC for 10 min
- pH
  - Can be used for serum treatment
  - Mycoplasma sensitive to ph shift
- HTST and UV
  - Do not work well for undiluted serum
  - Serum containing media often treated in these ways
  - Mycoplasma species have been shown to be sensitive to these treatments

According to Barbara

- One recent contamination event confirmed
  - Triple 0.1 filtered serum
  - Not Gamma irradiated

- Reinforces the ISIA suggestion to Gamma irradiate

Industry Issues

Industry Issues

- When is a 0.1 micron filter a 0.1 micron filter?
  - 0.1 filters are rated not calibrated
  - PDA chapter on filtration will be very helpful
    - Standardization of pressure critical
  - ISIA members will be made aware of PDA output
- Standardization of test methods
  - Not just for mycoplasma
- Development of better post-manufacturing treatment methods

And Now

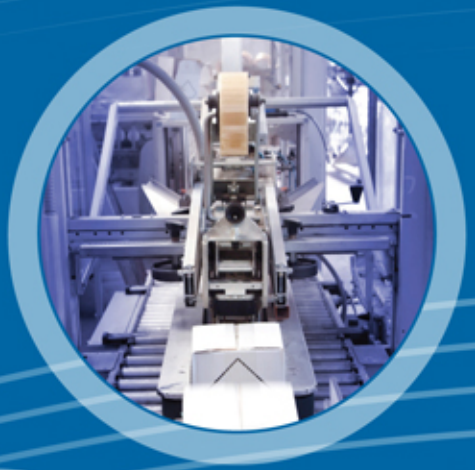
www.serumindustry.org

Peptones

For Something Completely different!!

Connecting People, Science and Regulation

Peptones: Problems and Solutions



Agenda

- What are peptones?
- What are they used for ?
- How are they manufactured?
- How does the manufacturing process impact adventitous agents?
- What can be done to further lower risk?

Childhood Memories

Peptones

- Any of various water-soluble protein derivatives obtained by partial hydrolysis of a protein by an acid or enzyme during digestion, and used in culture media in bacteriology
- Excellent natural sources of amino acids, peptides and proteins in growth media.
- Most often obtained by enzymatic digestion or acid hydrolysis of natural products, such as animal tissues, milk, plants or microbial cultures.

Peptones

- Raw materials may include
  - Bovine
    - Heart, lung and other components
    - Milk and milk derivatives,
    - gelatin
  - Porcine meat
  - Soy
  - yeast and grains.

Enzyme sources include

- Enzyme selection is based on substrate to be used
  - Animal organs
    - pancreatin (trypsin)
    - Pepsin
  - Fruits
    - Papaya (papain)
    - Pineapple (bromelain)
  - Proteases from bacterial, algal, fungal and yeast sources

What can peptones be used for?

- Animal cell culture for the production of monoclonal antibodies, therapeutic proteins, enzymes, etc
- Recombinant culture fermentations for the manufacture of therapeutic drugs, vaccines, etc.
- Insect and plant cell cultures for a variety of end products
- Specialized media for growing and expressing genetically modified micro- organisms

Adventitous agents

- Give the applications, similar concerns exist as for other animal derived materials in biopharma applications
- As ever, the control of adventitious agents includes sourcing of raw materials for prions, testing, cleaning/decontamination, filtration, heat, low pH and gamma irradiation for other agents.

Focus on Processing

Manufacturing Process

Raw material

Hydrolysis
Acid or enzyme

Stop
hydrolysis

Clean up

Pasteurization

Processing steps

- Initially, raw material proteins are solubilized in water at between 8–20% solids.
- If needed the proteins are pretreated with heat (up to 93˚ C), and acid or alkali to ensure solubilization
- Adjusted to appropriate pH (3.5–9.0) and temperature (38–65˚ C) prior to hydrolysis

Acid Hydrolysis

- Harsh process,
- Carried out at high temperature under pressure
- Attacks all peptide bonds in the protein substrate,
- Destroys or impacts some of the amino acids liberated.
    - Tryptophan
    - Cystine, Serine and Threonine
    - Asparagine and Glutamine

Acid Hydrolysis parameters

- Concentration and type of acid (hydrochloric acid or sulfuric acid)
- Temperature (250–280˚ F)
- Pressure (32–45 psi)
- Time of hydrolysis (2–8 h)
- Concentration of protein (50–65%)

Enzymatic hydrolysis

- More gentle process than acid hydrolysis
- Does not require the high temperature used for acid hydrolysis
- Specific peptide bonds broken by specific enzymes.
  - The resulting material from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths.

Pasteurization

- The product is typically pasteurized or heat treated to kill/ reduce the microorganisms.
- The term pasteurization is misleading because in most cases the temperatures used are much higher than standard legal pasteurization temperatures.
- Some manufacturers pasteurize the product multiple times.

Impact of Steps on Mycoplasma

Processing step	Heat	Effect
Solubilization	93°C	~~Mycoplasma~~ ?
Acid treatment	Up to 140°C	~~Mycoplasma~~
Enzyme treatment	38-65°C for hours	Mycoplasma??
Pasteurization	Above 72°C for 15 seconds	~~Mycoplasma~~

Ten Minutes at 65°C leaves no detectable mycoplasma

Processing steps highly likely to reduce mycoplasma contamination

So what can you do!

- Know your supplier and their process!!
  - Ask for data with regard to heat and timing
- Select less risky sources of raw material
  - BHI???
- Select animal free peptones if possible
  - Check history for animal components
- Is all else fails Gamma irradiate!
  - 25 Kgy gives 6 log reduction

Thank You!

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www.serumindustry.org