



U.S. PHARMACOPEIA
The Standard of QualitySM

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Standards for Ancillary & Process Materials – Protein A and Fetal Bovine Serum

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Expert Committees and Advisory Panels in Biologics & Biotechnology

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Biologics & Biotechnology Collaborative Group

Blood & Blood Products

Human Plasma

Heparin

Plasma Analytical

Vaccines & Virology

Vaccine Tests

Virology Tests

Immunology Tests

NAT

Viral Clearance

Viral Testing Plasma

Proteins & Polysaccharides

Bioassay analysis

Bioassay Development

Bioassay Validation

Monoclonal Antibodies

Protein A

Glycans

Enzymes

Cell, Gene & Tissue Therapies

Flow Cytometry

Test Kit Validation

Cell Therapy

Gene Therapy

Bovine Serum



Why worry about ancillary and process materials?

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USP 31

General Information / <1043> Ancillary Materials 403

<1043> ANCILLARY MATERIALS FOR CELL, GENE, AND TISSUE-ENGINEERED PRODUCTS

INTRODUCTION

A wide variety of reagents and materials, many of which are unique or complex, are required for the manufacture of cell, gene, and tissue-engineered products. These materials include plasma- or serum-derived products, biological extracts, antibiotics, cytokines, culture media, antibodies, polymeric matrices, separation devices, density gradient media, toxins, conditioned media supplied by “feeder cell layers”, fine chemicals, enzymes, and processing buffers. Many of these items are used to ensure the survival and promote the growth of certain cell populations, although their mechanism of action may not be entirely understood. Examples include fetal bovine serum (FBS) and various media supplements. Other items, such as highly purified cholera toxin, are introduced into the processing stream during manufacturing to exert a specific biochemical effect and are immediately washed out in subsequent processing steps to avoid unwanted toxicity at a later point. The finished biological products produced in such processes are often complex mixtures that, in some cases, cannot be completely characterized. Careful scrutiny of the materials used in manufacturing is necessary to prevent the introduction of adventitious agents or toxic impurities, as well as to ensure the ultimate safety, effectiveness, and consistency of the final product.

therapeutic entity. These risks to the quality and safety of the therapeutic product are often heightened with cell, gene, and tissue-engineered products, due to the limited ability to conduct extensive in-process and release tests. For example, lack of in-process holding steps or limited shelf life may create the need to administer the cell, gene, or tissue-engineered products before in-process or final-release testing results are available. In other cases, the scarcity of suitable donor tissue or the complex logistics in the transport of biological materials may limit the amount of material available for testing. To minimize these risks, whenever possible, it is necessary to implement rigorous material qualification and prudent application of manufacturing process controls.

Frequently, these novel therapeutic products are created using complicated biological processes. The AMs employed in these procedures may be selected primarily for their unique functional contributions or biological effects. Whenever possible, it is preferable to source AMs that are approved or licensed therapeutic products because they are well characterized, have an established toxicological profile, and are manufactured according to controlled and documented procedures. Conversely, the AM may be intended “for research use” and may, therefore, lack the level of qualification necessary for use in the production of a therapeutic product. In either case, the manufacturer of the cell, gene, or tissue-engineered product should develop comprehensive and scientifically sound qualification plans to ensure the traceability, consistency, suitability, purity, and safety of the AM. In cases where AMs are products approved for use for therapeutic purposes, the level of qualification will probably be less extensive than that for a material intended for research purposes. However, their suitability in the manufacturing process will still need to be established when the AM is being used beyond the scope of its intended use or labeling. The purpose of this chapter is to provide guidance in developing appropriate qualification programs for AMs employed in cell, gene, and tissue-engineered product manufacturing.



<1043> says...

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“Careful scrutiny of the materials used in manufacturing is necessary to prevent the introduction of adventitious agents or toxic impurities, as well as to ensure the ultimate safety, effectiveness and consistency of the final product.”



Fetal Bovine Serum Advisory Panel- Scope

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- ◆ FBS and other types of serum are important components of biotechnology industry
- ◆ There is a lack of standards to measure the quality of these products
- ◆ Formation of a USP Panel to work on setting standards for Bovine Serum Products.
- ◆ The FBS panel reports to the BB CGT EC and BB VV EC
- ◆ Scope of the panel is to address issues related to:
 - ▶ Characterization of bovine serum products
 - ▶ Requirements for a standard for FBS products
 - ▶ Functionality tests for FBS



<1024> *Bovine Serum* Chapter Status

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- ◆ Chapter Outline
 - Introduction
 - The uses of Fetal Bovine serum
 - Serum Harvesting and Collection
 - BSE/TSE risks
 - Testing and control of Adventitious Agents
 - Characterization of Bovine Serum
 - Conclusions
- ◆ Timeline
 - ▶ Submitted to PF
 - ▶ Publication in PF 34(3) – May/june 2008
 - ▶ 90 days public comment period
 - ▶ Expected Official with USP32 1S



Fetal Bovine Serum (FBS) Specifications – Test Chapter Development

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- ◆ Definition
- ◆ Packaging and Storage
- ◆ Labeling
- ◆ USP Reference Standards
- ◆ pH <791>
- ◆ Osmolality <758>
- ◆ Total Protein <1057>
- ◆ Endotoxins <85>
- ◆ Sterility <71>
- ◆ Adventitious Agents
- ◆ Identification
 - ▶ Electrophoresis profile
 - ▶ Ouchterlony (double diffusion)
 - ▶ RID
- ◆ Specific Tests
 - ▶ Hemoglobin levels <851>



<90> *Bovine Serum Quality Attributes and Functionality Tests*

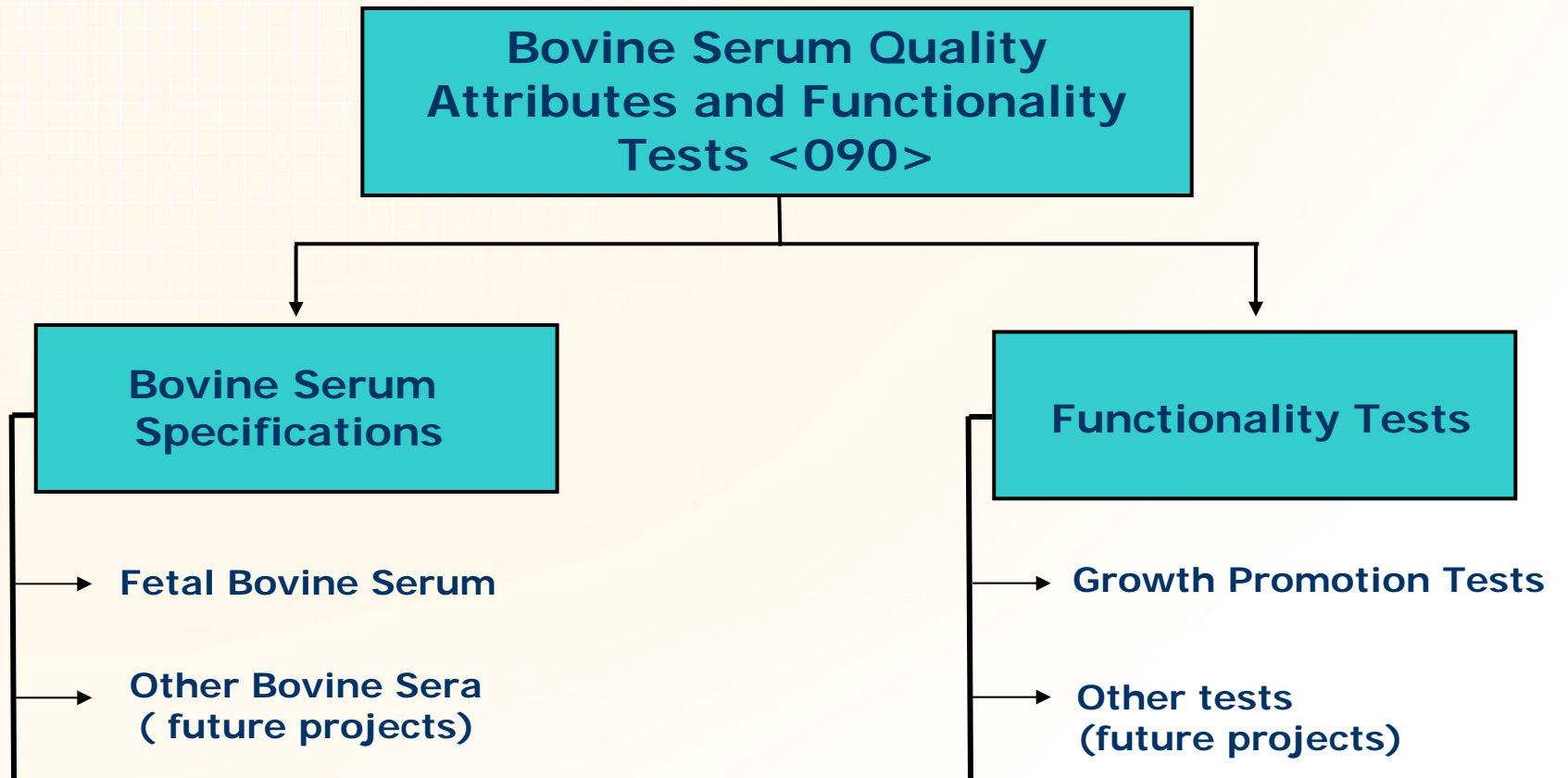
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- ◆ Draft Chapter to be finalized based on method development and compendial verification studies
- ◆ Tentative publication date is PF34(4) - July-August 2008
- ◆ 90 days for public comments period
- ◆ Expected Official with USP32 2S



Structure of the <090> Chapter

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FBS Specifications

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- ◆ Osmolality: 280-360 mOsm/Kg
- ◆ Total Protein: 30-45 mg/mL
- ◆ pH: 7-8
- ◆ Endotoxins: <10 Units/mL
- ◆ Hemoglobin levels <30 mg/dL
- ◆ Sterility: Meet requirements under <71 >
- ◆ Electrophoresis profile (normal, compared to RS?)
- ◆ RadialImmunoDiffusion (RID) (normal, compared to RS?)



FBS Specifications

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- ◆ Data collected from surveying suppliers, shows variability
- ◆ Ranges were chosen to promote harmonization between manufacturers, but also harmonization with the European Pharmacopeia (EP)
- ◆ Lack of characterized standard used with the tests method for FBS
- ◆ Need to conduct independent study for verification and validation of the methods



Bovine Serum samples for Compendial Verification and methods development

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| Manufacturer | Fetal | Newborn | Calf | Adult |
|--------------|---------|---------|------|-------|
| #1 | US | US | US | |
| #2 | US | US | | NZ |
| | AUS | | | |
| | NZ | | | |
| | Low IgG | | | |
| #3 | US | US | US | NZ |
| | AUS | | | |
| | NZ | | | |
| | Low IgG | | | |
| #4 | US | US | US | US |
| | AUS | NZ | AUS | |
| | NZ | | | |



FBS Compendial verification (USP methods)

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◆ Fetal Bovine Serum (FBS) Samples

- ▶ 1- Supplier 1
- ▶ 2- Supplier 2-a
- ▶ 3- Supplier 2-b
- ▶ 4- Supplier 3-a
- ▶ 5- Supplier 3-b
- ▶ 6- Supplier 4

◆ Methods

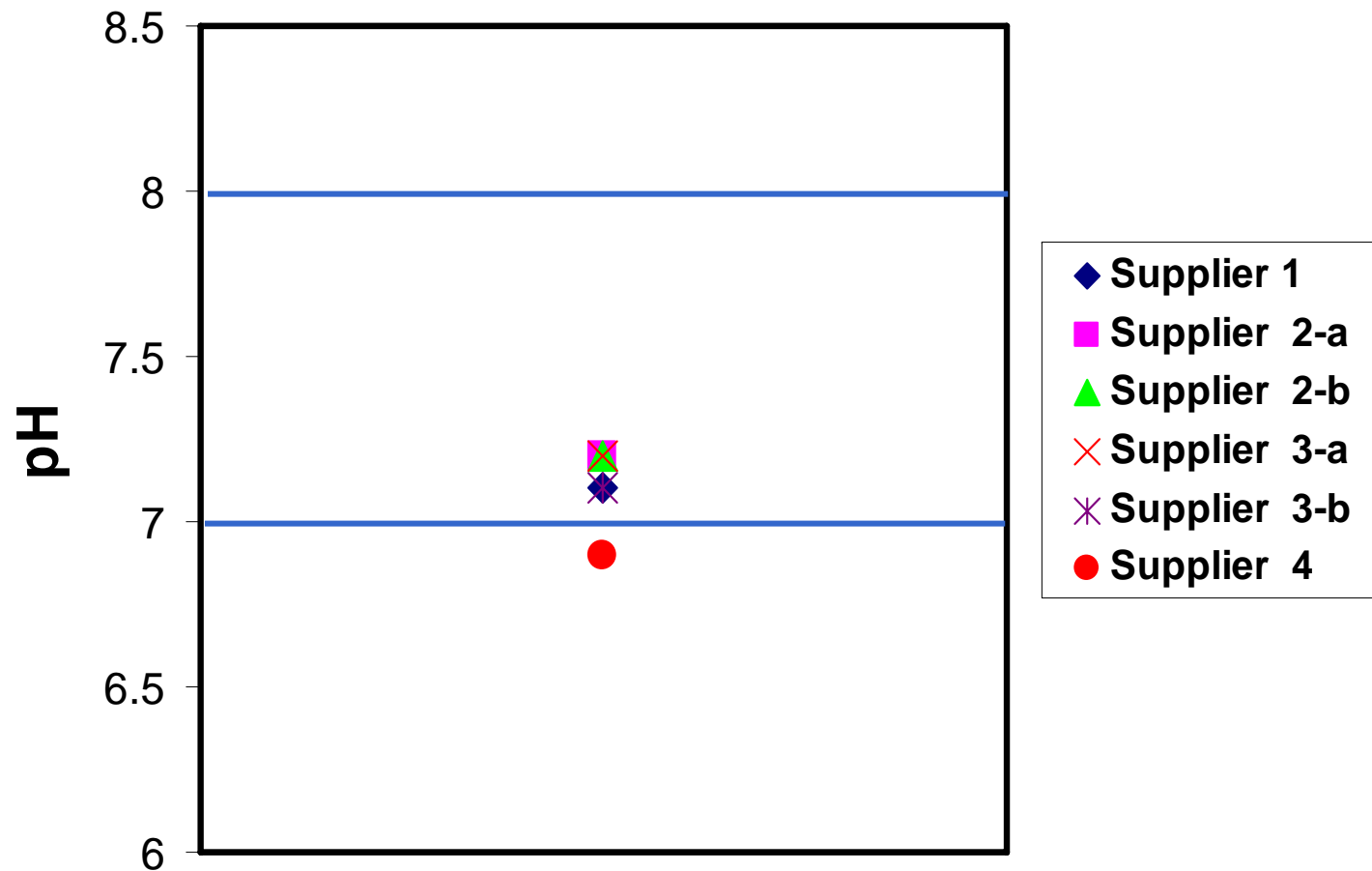
- ▶ pH <791>
- ▶ Osmolality <758>
- ▶ Total protein <1057>
- ▶ Hemoglobin content <851>
- ▶ Endotoxins <85>



pH

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pH (7.00-8.00)

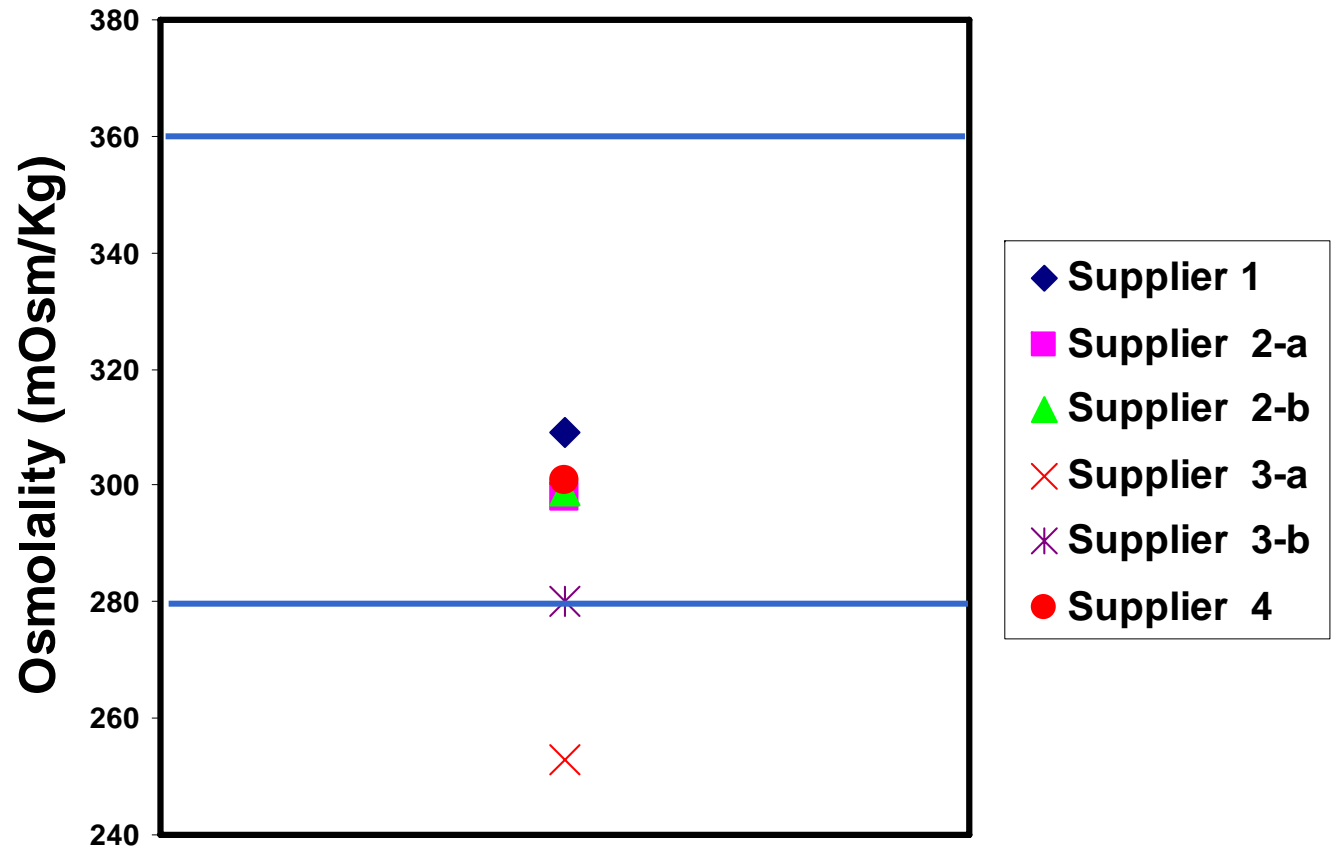




Osmolality

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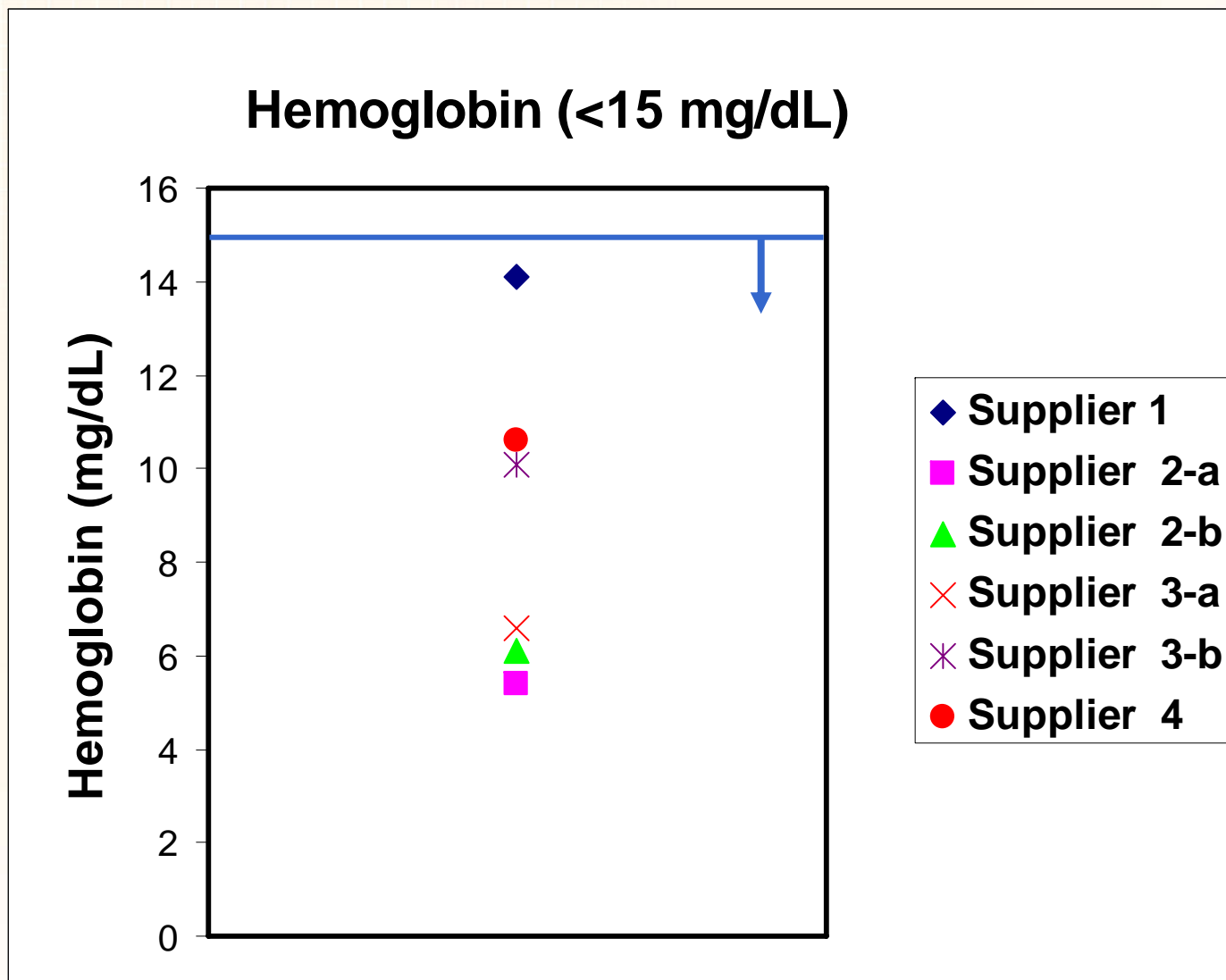
Osmolality (280-360 mOsm/Kg)





Hemoglobin

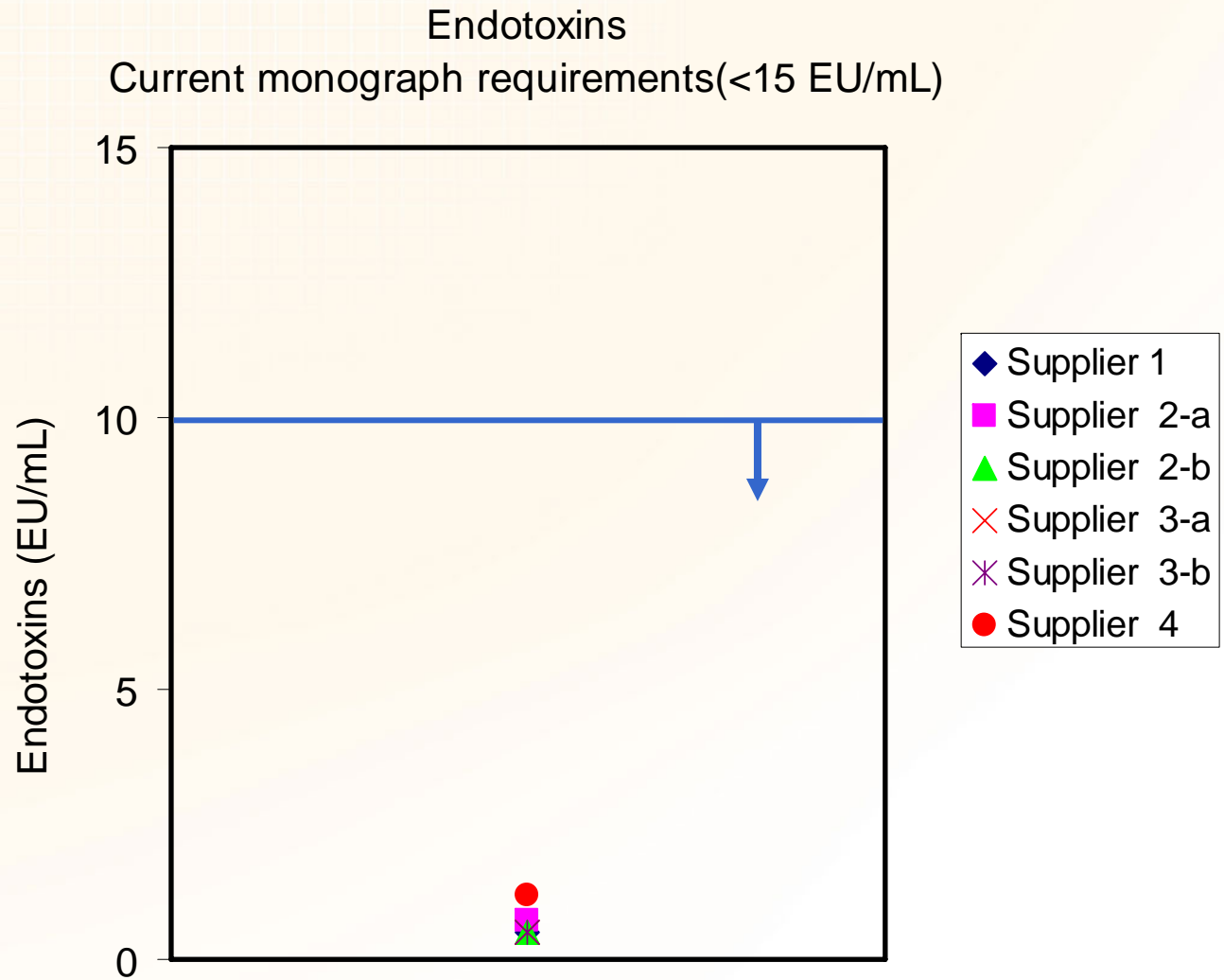
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Endotoxins

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Compendial Verification- Conclusions

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- ◆ Very little variability for pH and Endotoxins
 - ▶ Levels of endotoxins are very low compared to data from suppliers

- ◆ Variability for total protein between suppliers and between methods
 - ▶ Do we need to consider other methods?

- ◆ Variability for Hemoglobin levels but all results are below the limit set at <15 mg/dL

- ◆ Do we need to consider revising ranges for these analytes?



FBS Methods development

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- ◆ Methods to develop
 - ▶ Electrophoresis profile
 - ▶ Radial ImmunoDiffusion (RID)
- ◆ Fetal Bovine Serum (FBS) Samples
 - ▶ FBS from different suppliers
 - ▶ Control Human Serum (for Electrophoresis)
 - ▶ Horse Serum



USP Study- Conclusions

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- ◆ USP study shows some variability but overall consistency between suppliers for FBS specifications
- ◆ Results provide good basis for developing standards for FBS
- ◆ Highest quality of FBS is needed by end users and demanded by regulators (under cGMPs)
- ◆ USP Strategy:
 - ▶ Finalize the methods development
 - ▶ Move the monograph specifications to the <090> chapter
 - ▶ Identify a good reference material



Protein A

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- ◆ Protein A is used in the purification of most monoclonal antibodies
- ◆ Leakage of Protein A is monitored via an ELISA
- ◆ The protein A ELISA is either developed by each antibody manufacturer or a commercial kit is used
- ◆ Of concern is the lack of a universal standard
- ◆ The objective is to provide a standard to aid in an accurate determination of the amount of residual Protein A
- ◆ At low levels Protein A is not considered to be of a concern for safety



Protein A Ad Hoc Advisory Panel

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- ◆ Reports to the B&B Proteins and Polysaccharides Expert Committee
- ◆ Chair: Michael Mulkerrin, PhD, Oncomed
- ◆ Anita Szajek, PhD, USP Liaison
- ◆ Walter Hauk, PhD, USP Statistician

- ◆ Panel Members:
 - ▶ James Bingham, PhD, Amplimmune
 - ▶ Tomas Björkman, PhD, GE Healthcare
 - ▶ Russell Hart, PhD, Luminos (Assay Designs)
 - ▶ Kenneth Hoffman, PhD, Cygnus Tech
 - ▶ Anders Larsson, PhD, Immunsystems AB
 - ▶ Jay Madan, Reliance
 - ▶ Ariane Marolewski, PhD, Repligen
 - ▶ Victor Van Cleave, PhD, Viral Antigens
 - ▶ Helen Wood, Millipore



Protein A Advisory Panel

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“...It is the purpose of this advisory panel to develop a monograph for protein A that defines the standard of quality for this very common process material. Also, an associated reference standard should be developed that will provide a crucial common measure for the residuals-tests used to demonstrate the removal of protein A from the drug substance...”



Protein A Chapter and Reference Standard

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- ◆ Objective is to develop the a Chapter and the associated Reference Standards for the Protein A molecules that are used in the purification of Monoclonal Antibodies
- ◆ Intended for two uses
 - ▶ Standard for demonstration of quality attributes
 - ▶ Standard for the Protein A impurity ELISA
- ◆ Specifications for each available Protein A construct in USP Chapter <130>



Protein A Constructs

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| Protein A | rProtein A | rProtein A, C-Cys | rProtein A, B4, C-Cys |
|---------------------------------------|--|---|--|
| 46.8 kDa | 44.6 kDa | 34.3 kDa | 26.7 kD |
| <i>Staph. aureus</i> | <i>E. coli</i> | <i>E. coli</i> | <i>E. coli</i> |
| 5 IgG binding domains (E, D, A, B, C) | 5 IgG binding domains; different N-terminal sequence than Natural PA | Lacks C-term membrane binding AA sequence; addition of a Cys for immobilization | B-domain: alkaline-stabilized by site-directed mutagenesis and tetramerized; addition of a Cys |
| Free of animal component | Free of Animal Components | Free of Animal Components | Free of Animal Components |



Major Activities to Date

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- ◆ Inception of the committee and recruiting the membership - June 2005
- ◆ First Committee meeting August 2005
- ◆ Submission of draft monographs - October 2005
- ◆ Workshop - 18 October 2005
- ◆ Methods Development- June 2006
- ◆ Agreement on the specifications - July 2006
- ◆ Reference standard fills completed – December 2006
- ◆ Monographs submitted to PF – Jan 2007
- ◆ PF publication – May 2007



Protein A Chapter Development

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Content of the Chapter <130>:

- ◆ Types of testing
 - ▶ Identity
 - ▶ Purity
 - ▶ Protein Concentration
 - ▶ Functional assay
 - ▶ Impurities
- ◆ Testing protocols
 - ▶ SOPs provided by the sponsors
 - Assay development required



Collaborative Study

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- ◆ Demonstrate the applicability of the reference standards for its intended use in the Protein A ELISA
 - ▶ ELISA kits: cross-platform variability
 - ▶ Inter-laboratory variability
 - ▶ Plate effect
 - ▶ Appropriateness of USP Protein A reference standard configuration



Protein A Chapter

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- ◆ Chapter <130> Protein A Quality attributes
- ◆ Published in Pharmacopeial Forum 33(3)
- ◆ Will become official in USP 31 NF26
- ◆ Upon Approval of B&B Proteins and Polysaccharides Committee reference chapter will be published
- ◆ Reference Standard will be made available through USP, currently in collaborative testing
- ◆ Standard Pharmacopeial Assay for protein A constructs will be published in USP <131> Residual Protein A Testing
 - ▶ ELISA Collaborative study



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Thank You