INTRODUCTION

PDA Workshop on Mycoplasma Contamination By Plant Peptones

Entering into the 21st century, the regulatory agencies and the pharmaceutical industry developed effective risk management programs to minimize the risk of bovine spongiforme encephalopathy (BSE) contamination in the manufactured biopharmaceuticals (e.g., recombinant DNA-derived proteins and monoclonal antibodies). Appropriate quality risk management programs have been instituted by (1) identifying the problem, (2) avoiding high risk biologically-based raw materials, (3) developing a close working relationship between the vendor of the biologically-based raw materials and the purchasing manufacturer, and (4) additional treatment and/or testing of these raw materials as needed upon QC receipt. While not eliminating the risk of BSE, these risk management programs have been effective in minimizing the risk, so that it can be proudly stated that no patient has been infected by the BSE agent due to the manufacturing process of a biopharmaceutical.

One part of the BSE risk management program has been to go 'vegetarian' with the selection of our raw materials. "We are safer using plant-derived raw materials rather than material derived from animal/bovine sources" has been the operating principle of the pharmaceutical industry. But did we become complacent in our concern about adventitious agents from these plant-based raw materials?

It all started with a comment on a FDA website entitled 'Questions and Answers on Current Good Manufacturing Practices, Good Guidance Practices, Level 2 Guidance: Production and Process Controls' (*www.fda.gov/cder/guidance/cGMPs/default.htm*) in August 2004:

A firm has multiple media fill failures. They conducted their media fills using TSB (tryptic soy broth) prepared by filtration through 0.2 micron sterilizing filter. Investigation did not show any obvious causes. What could be the source of contamination?

A firm recently had multiple media fill failures. The media fill runs, simulating the filling process during production, were conducted inside an isolator. The firm used TSB (non-sterile bulk powder) from a commercial source, and prepared the sterile solution by filtering through a 0.2 micron sterilizing filter. An investigation was launched to trace the source of contamination. The investigation was not successful in isolating or recovering the contaminating organism using conventional microbiological techniques, including the use of selective (e.g., blood agar) and nonselective (e.g., TSB and tryptic soy agar) media, and examination under a microscope. The contaminant was eventually identified to be *Acholeplasma laidlawii* by using 16S rRNA gene sequence. The firm subsequently conducted studies to confirm the presence of *Acholeplasma laidlawii* in the lot of TSB used. Therefore, it was not a contaminant from the process, but from the media source.

Acholeplasma laidlawii belongs to an order of mycoplasma. Mycoplasma contain only a cell membrane and have no cell wall. They are not susceptible to beta-lactams and do not take up Gram stain. Individual organisms are pleomorphic (assume various shape from cocci to rods to filaments), varying in size from 0.2 to 0.3 microns or smaller. It has been shown that *Acholeplasma laidlawii* is capable of penetrating a 0.2 micron filter, but is retained by a 0.1 micron filter (see Sundaram, *et al.*). *Acholeplasma laidlawii* is known to be associated with animal-derived material, and microbiological media is often from animal sources. Environmental monitoring of mycoplasma requires selective media (PPLO broth or agar).

Resolution:

For now, this firm has decided to filter prepared TSB, for use in media fills, through a 0.1 micron filter (note: we do not expect or require firms to routinely use 0.1 micron filters for media preparation). In the future, the firm will use sterile, irradiated TSB when it becomes available from a commercial supplier. (Firm's autoclave is too small to permit processing of TSB for media fills, so this was not a viable option.) The firm will continue monitoring for mycoplasma and has revalidated their cleaning

procedure to verify its removal. In this case, a thorough investigation by the firm led to a determination of the cause of the failure and an appropriate corrective action.

Mycoplasma contamination is no stranger to the biopharmaceutical industry. Reported cases of mycoplasma contaminations have affected upstream biological processes (i.e., cell culturing). What was a surprise was the FDA-reported case example of mycoplasma contamination in a downstream manufacturing operation without the presence of cells, specifically during a media fill study. Manufacturers have switched to non-animal-derived raw materials, such as phytones or soy peptones, to avoid any possibility of inadvertent BSE introductions from animal-derived raw materials. According to Barbara Potts, Ph.D., Director of Virology, Quality Control, Genentech, "We thought that our problems would all go away when we switched over to plant-derived materials. Much to our surprise, a different adventitious agent popped up as a major concern!"

The result was a PDA workshop on 'Mycoplasma Contamination by Plant Peptones' held in September 2005. Using an approach that was effective in managing the BSE concerns, the workshop brought together representatives from the pharmaceutical industry, regulators, vendors and academia to obtain a better understanding of the mycoplasma contamination in the raw materials, especially plant-derived materials.

The following is an overview of the workshop proceedings:

Leonard Hayflick, Ph.D., Professor of Anatomy, University of California, San Francisco (world's expert on mycoplasma) demonstrates that "Mycoplasma can be found almost anywhere. Just find an insect, plant or animal species that has not been studied yet, and you can probably discover a mycoplasma that you can name yourself." Mycoplasmas are characterized by their small size (about 0.2 microns compared to bacteria of about 5 micron or larger), lack of a cell wall, and difficulty to culture in the absence of cells.

Patricia Hughes, Consumer Safety Officer, CDER, Office of Compliance, FDA, discusses the use of TSB in an aseptic fill/finish manufacturing facility that the mycoplasma problem first surfaced. Tryptic Soy Broth (TSB), also known as Soybean-Casein Digest Medium (SCDM), Trypticase Soy Broth, Tryptone Soya Broth and many other names, is suitable for the culture of both fungi and aerobic bacteria, and as such is used in process simulations to validate aseptic processing (i.e., media fills).

The industry experience with encountering mycoplasma contamination, especially during media fill simulation of an aseptic process, is reviewed by **Ivar Kljavin**, Ph.D., Senior Manager, Quality Control, Genentech; **Pranhitha Reddy**, Senior Principal Scientist, Amgen; and **Cynthia E. Romera-Arroya**, Ph.D., Ortho Biologics. Mycoplasma contamination in plant-derived raw materials appears to be more common than anticipated.

What are the possible sources of mycoplasma in TSB and other plant-derived materials? The sources could be either animal-based or plant-based: (1) animal-derived enzymes used in the hydrolysis of the plant material, (2) animal-derived contamination introduced during the growth or harvesting of the plant material such as animal manure sprayed on plants, and (3) Phytoplasmas (mycoplasma-like organisms) that cause diseases in plant species. Vendor recommendations for either removing or inactivating mycoplasma in plant-derived raw materials are provided by **Christopher Wilcox**, Technical Services Manager, Kerry Bio-Science; Marc Glogovsky, Senior Eastern Regional Field Sales Specialist, EMD Chemicals; **Jerold Martin**, Senior Vice President, Scientific Affairs, Pall Corp.; **Maik W. Jornitz**, Group Vice President, Sartorius North America; and **Suraj Baloda,** Group Manager, Millipore Corp.

Waiting 28 days for the standard FDA Points to Consider mycoplasma test results has hindered early detection of mycoplasma contamination. New, more rapid test methods for detecting mycoplasma contamination using polymerase chain reaction (PCR) approaches are described by **Barbara J. Potts**, Director of Virology, Quality Control, Genentech; and **Audrey Chang**, Ph.D., Senior Director, Biologic, BioReliance, Invitrogen Bioservices.

The proceedings of this workshop will serve as an invaluable resource for everyone in the biophar-maceutical industry. It provides sound approaches to developing an effective quality risk management program for minimizing the risk of mycoplasma contamination in the manufacturing processes. It also provides a foundation for further discussion between academia, manufacturers, vendors and regulators on this important subject.

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