Non-compendial Microorganisms in the QC Microbiology Lab

By Dr. Scott Sutton

The QC microbiology lab has, as its primary responsibility, the reporting of accurate and timely information on the microbiological quality of the company's facility and products. This is normally accomplished by environmental monitoring of the facility's area and utilities, and by in-process and finished product testing of the products. Let's take a look at the first consideration.

Biological contamination of products can come from diverse sources – an example of this is provided in the figure to the right (larger diagram on Page 4). What is important to remember about these contaminants is that we can only track and trend those organisms that we are able to recover. This may require the use of additional media beyond the accustomed TSA and SDA.

TSA (Trypticase soy agar, also known and soybean-casein digest agar) is an excellent choice of media for recovery of a wide range of microorganisms (Leavitt 1955). However, it is not appropriate for recovery of all potentially interesting microorganisms. For example, many have reported that Reasoner's (1985) R2A agar is superior to TSA in pharmaceutical water systems (Sundram 2001, Jhamb 2002). Similarly, Plate Count Agar (PCA) may also recover less microorganisms than R2A (Massa 1998). It would therefore be well-advised to check recovery of microorganisms from your water system on both different media, validating your recovery method to the bioburden in your specific water system[1].

Water testing is not the only example of a situation where different media might be important. Another might well be testing of shampoos which may be susceptible to contamination and growth of a small number of different halophilic organisms. Many of these will not grow well on TSA and prefer growth on a high-salt medium like Mannitol Salt Agar. In this case also the growth promoting properties of the media should be confirmed with species specifically found to be of interest in the facility.

The presence of yeast and mold might also be important – especially if water damage is suspected in the facility. SDA (Sabouraud Dextrose Agar) is a selective medium for aciduric microorganisms with a pH of 5.6 (Sabouraud 1892). While this will be primarily yeast and mold, there are some aciduric microorganisms which will grow through on this medium as well. If there is a particular species of microorganism that is contaminating your product, it might be wise to ensure the growth promoting properties of your SDA against this microorganism.

The point of this discussion is that we are responsible to ensure that the testing performed on the environment, and on the in-process materials and finished products, are relevant to determine the true state of microbiological quality. This may require unusual media, and may also require Quality Control testing of media used with non-compendial species of bacterial to demonstrate growth promotion of the media used.

The question of using a novel species of microorganism to challenge your media growth promotion at manufacture and on stability (Longstaff 1997) is one that we should be considering carefully, especially when the microorganism is one that is associated with past problems in the facility (difficult to eradicate) or the product (sterility failures or over-growth in non-sterile products).

Turning to the second primary function of the QC microbiology – providing accurate and timely information on the company's products. The main tests that we will be performing include bioburden tests of incoming raw materials and in-process controls, and finished product testing. The bioburden test will have the same growth promotion concerns described above. Finished product testing might include Sterility testing, microbial limits testing and antimicrobial effectiveness testing. Sterility testing is well-defined in the compendia, as is microbial limits testing. However it might be wise to challenge the media with specific species isolated from past investigations into product issues as a precaution.

Antimicrobial effectiveness testing is a different concern. The point to the compendial test is to provide a reproducible measure of the biological activity of the product's preservative system using a defined battery of challenge organisms (Sutton and Porter 2002). Its purpose is not to demonstrate activity against all potential microorganisms that the product might possibly find in the field. Given a wide enough distribution, any product may show failure against specific species of microorganisms – these might make excellent additional challenge organisms for the Antimicrobial Effectiveness Test (AET).

Another reason to look to specific, non-compendial microorganisms for QC microbiological testing and Quality control has nothing to do with the actual challenge to the product or the facility, but rather to regulatory concerns. There has been a great deal of concern expressed recently over *Burkholderia cepacia* from the Agency (Torbeck 2011; but also see Sutton 2012 for response). Similarly, *Bacillus cereus* has become a concern in some cosmetic microbial quality discussions with the Agency (personal communication). It might therefore be prudent to maintain a stock of these organisms (as appropriate) for use in antimicrobial efficacy testing and growth promotion testing for media.

This brings us to the issue of maintenance of the stock cultures. While almost every company could benefit from having a variety of non-compendial microorganisms for specialized testing, setting up

the culture collection can be a challenge for many. The most direct method, if this is to be done in-house, is through the use of a seed lot culture method with freezing of the strains at ≤70ºC (http://www.microbiologynetwork.com/seed-lot-culture-technique.asp). This allows the long-term storage of the microorganisms. The establishment of a sustainable culture collection is costly in terms of space and labor, and will require a GMP tracking system to follow the lineage, purity and QC testing for each working culture.

A second method to maintain a culture collection would be to contract the work off-site to a company that specializes in this type of work. Of course, all of the same GMP expectations would be in place for this contract facility (Approved Vendor, current audit, etc) and given the specialized nature of the work being performed it would be wise to include someone with microbiological knowledge in the audit team. However, once qualified, contracting this "unusual" culture maintenance work out can be cost-effective and convenient

Summary

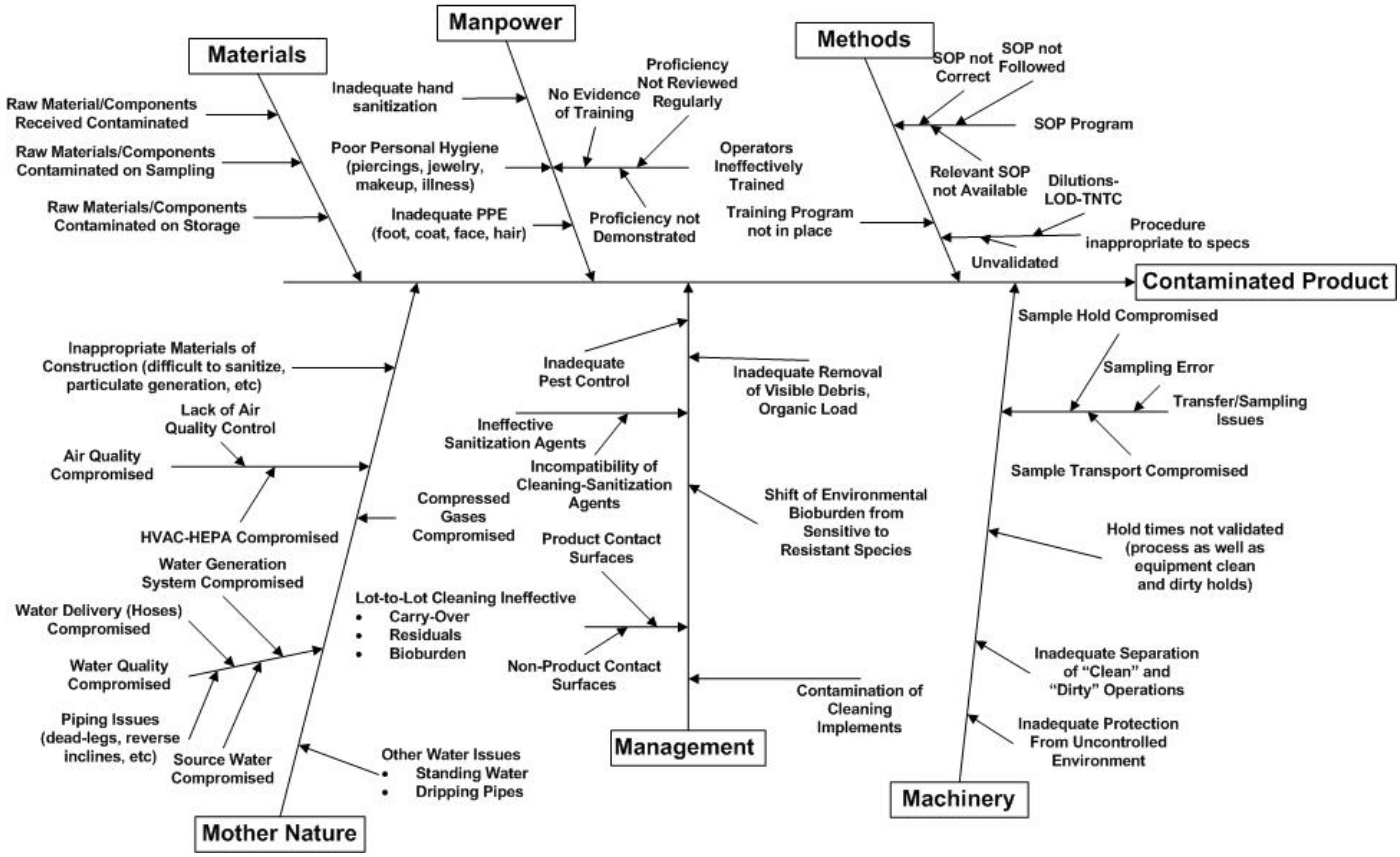
There are compelling reasons for the maintenance of non-compendial microorganisms in the QC microbiology lab. These include challenging of standard media with unusual species, qualifying selective media with unusual species of microorganisms, or performing standard tests (eg the AET) with an expanded battery of microorganism species. The laboratory can choose to develop an in-house culture collection or take advantage of contract facilities that offer this service.

Biography:

Dr. Scott Sutton earned his B.S. in Genetics from the University of California at Davis, and his Masters and Ph.D. in Microbiology from the University of Rochester (Rochester, NY). After an NIH postdoctoral fellowship at the Medical College of Virginia (Richmond, VA), he went to work for Bausch and Lomb (Rochester, NY) until 1994 when he accepted a position at Alcon Laboratories (Fort Worth, TX). Dr. Sutton left Alcon Laboratories in 2004 as a Director in the R&D division to accept a position as Pharma Consultant (Microbiology) with Vectech Pharmaceutical Consultants, Inc which he left in 2009 as Sr. Director, Microbiology Services.

Scott Sutton is the Principal of Microbiology Network, Inc a company he started in 1996 as a means to encourage training and communications within the microbiological community. With over 70 publications and hundreds of presentations, he is a recognized consultant and trainer with emphasis in GMP, investigations, Environmental Monitoring and contamination control (both Aseptic manufacturing and non-sterile production facilities) as well as microbiology laboratory audits and operations. Dr. Sutton is an active author and speaker for the industry, supports ASM, PCPC and PDA, and has served with the USP Analytical Microbiology Committee of Experts since 1993.

Cause & Effect Diagram for Potential Causes of Contaminated Product "6M"



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Footnote: [1] It is also worth noting that there is evidence arguing that the pour-plate method of plating results in a reduced recovery compared to membrane filtration – plating method also is important!