



## EFFECTIVE ASEPTIC MATERIAL TRANSFER = LESS QUALITY FAILURES

A SILICONE-BASED ASEPTIC SAMPLING, INOCULATION AND TRANSFER SYSTEM FOR CONTAMINATION CONTROL IN PHARMACEUTICAL PROCESSING HAS BEEN RECENTLY INTRODUCED.

Your pilot-scale bioreactor is in full process with the recombinant protein project of an important new client. The specialised media ingredients were expensive. Gradually, the rate of base addition creeps beyond the rate you expect and your suspicions are aroused. You pull a sample and examination under a microscope confirms your fear - a strange microbe has found its way into the process and has taken over. All you can do is kill the broth and flush it into the waste system, losing the amount of time and materials in which you've invested. Then you start the

This scenario is a nightmare for a bioprocessing pilot plant operator - a situation operators take tedious steps to avoid. Operators follow an integrated quality control programme that begins well before the first vial of inoculum is opened and continues until the product is delivered and accepted.

### INDIVIDUAL STEPS TO QUALITY

Standard quality control systems are followed in all competent pilot plant operations. But it's not the entire systematic process that helps ensure quality. It is the individual steps that make up the programme

important steps in any quality programme is the aseptic transfer of small volume or low flow rate materials into the bioreactor while the process is in progress. Yet, quite often, plant operators have not taken the time to truly understand why this step is so important. Quality Management, Inc., (QMI) has built an international business based on the importance of this one step in the quality process.

Based in St. Paul, Minnesota, QMI is a manufacturer of products and product systems that help ensure aseptic transfer and inoculation during micro-

author, a microbiologist, QMI originally manufactured and marketed aseptic transfer and inoculation systems for the food processing (particularly the dairy) industry. Food processing companies throughout the world have used the staple QMI product, a single-needle port made up of non-coring rubber or silicone, protected by a metal base. Users of the QMI product in the food processing industry include Yoplait Yogurt, Dannon and Nestlé's.

### SAFE SEPTUM

In 1993, QMI designed transfer and inoculation products specifically for microprocessing and pharmaceutical applications, as well as for the brewing and distilling industry. The product, which was named the Safe-Septum™, was introduced to these industries in rubber and silicone-based options. The Safe Septum is now in use at such microprocessing facilities as Lifecore Biomedical and the Biological Process Technology Institute at the University of Minnesota.

Aseptic materials transferred into the bioreactor vessel during a process run include acid and base for pH control, anti-foam agents, nutrient solutions, chemical solutions for biotransformation or as a part of the process, and gases. Cultured inoculum transfer obviously must be done with the same care as any of the other sterile processes. Samples of the materials in process must also be drawn out of the bioreactor with the same level of attention to avoid contamination.

### CONFIGURING LARGE VOLUME TRANSFER SYSTEMS

The transfer of these materials and the appropriate transfer system varies according to the volume of material and flow rate necessary. Aseptic materials required in large volumes, for example a filter-sterilised nutrient solution, are usually moved in sanitary lines that are sterilised in place and used once. Configuration of large volume transfer systems for sterile or pure culture materials into and out of bioreactors receives significant attention during equipment and process design.

Widely accepted systems of sterilisation that plant operators have used for years include the steam-in-place



the materials needed for fixed or flexible lines - and pumps for moving large volumes. When these systems are operated according to manufacturers' suggested procedures, their performance can be monitored. They typically work well and allow for the aseptic transfer of large volumes into and out of bioreactors during process operation.

Materials that are required in small volumes or at low flow rates can be transferred using small lines that may be connected using an aseptic technique after the bioreactor vessel is sterile and/or while the process is running. These small volume addition ports, when properly designed and operated, have also become standardised in the industry.

Surprisingly, the techniques for transferring small volume or low flow rate materials are given little attention by plant operators. Although the transfer must be quick and efficient, it must also be completed using the latest technologies to help ensure the method is aseptic. The latest technology in aseptic transfer of small or low flow rate materials with bioreactor processing is the QMI Safe-Septum.

## UNDERSTANDING YOUR OPTIONS

Currently, the three most commonly used configurations in pilot-scale bioreactor facilities are miniature sanitary couplings, spring-loaded quick-connect fittings, and septum/needle combinations.

### Sanitary fittings

Many pilot plant operators prefer the miniature sanitary fitting because it looks like larger manufacturing systems. Yet this configuration is probably the least convenient type of fitting to connect while a process is running. An operator must open a sterile port with one of these fittings while keeping a gasket in place, positioning the mating sterile connector, and clamping the connection together. Even the extremely cautious (and coordinated) technician has trouble avoiding the contamination of surfaces that come in contact with the process liquids being added or removed. This configuration, which has to be steam-sterilised after the connection is made, is dangerous. The tortu-

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construction of the addition vessel being attached may not allow for adequate sterilisation of the connection area. Small sanitary fittings are most appropriate when the connection is made before the process vessel is sterilised. The connection can then be sterilised along with the rest of the process equipment and reliable aseptic transfer of materials can be accomplished.

### Quick-connects

A more convenient configuration is that of the spring-loaded quick-connect fittings. An aseptic connection is made when the female connector is attached to the addition vessel and the male connector is configured on the bioreactor vessel to be covered and independently sterilised. The sterilisation of the male connector is especially useful if more than one connection must be made to a single addition port during the process run. Yet, when using the quick-connections, you must plan ahead. The need for an additional port may arise and be unforeseen. If you are out of ports, your process may be over before your product is complete.

### Septum/needle systems

Many plant operators consider the septum/needle combination method to be the most convenient and the lowest cost option. In the past, fresh rubber septums were installed for every run and they were sterilised with the wipe of an appropriate sanitising agent. When they were penetrated with a sterile needle, most operators assumed the transaction was as aseptic as it could possibly be.

Because of the QMI Safe-Septum, the older septum/needle systems have become antiquated, especially when you consider the increasingly stringent quality requirements of modern bioprocesses and the high cost of bioprocessing materials and labour - all of which are compromised when older systems are used. Plant operators have begun to recognise the shortcomings of the older systems, but have relied on them anyway, even though

low cost and convenience. The author, in working with the Biological Process Technology Institute at the University of Minnesota, also recognised the need to refine this choice of aseptic transfer method so that quality requirements were met, costs were kept low, and no materials or labour were lost because of failed or contaminated processes.

The main sources of failure found with the older septum/needle designs are surface contamination and the inability of the septum to reseal after every puncture by a needle.

## PRESSURES OF STEAM STERILISATION

The older design requires support for the rubber to allow it to withstand the pressures of steam sterilisation. The perforated metal used to support the rubber septa creates the perfect hiding place for microbial contaminants, protecting them both from the heat and the contact with the sanitising agent used to sterilise the septum before it is penetrated with a needle. Thus, contaminants can escape from the very things that are meant to kill them. This means that the incoming needle may transfer live microbial contaminants into the bioreactor vessel and lead to process failure.

The QMI Safe-Septum is designed to withstand vessel pressurisation without the supplementary support. The only potentially non-sterile surface that comes in contact with the needle during the transfer of material is a smooth polymer sheet that is easily sanitised before it is pierced. Because the interior of the septum cartridge is never opened, it remains sterile as packaged, and the surface in contact with the bioreactor interior is sterilised along with the rest of the interior surfaces at the beginning of the transaction.

The sealing problem with older septum designs is often caused by the coring of the rubber materials resulting from the use of larger needles. After the needle is removed from the penetrated septum, typically the hole it makes does not reseal. The result can be

exterior of the vessel at this hole site, caused by positive pressure. Alternatively, the rubber's failure to reseal can cause the outright transfer of contaminated plant air into the vessel interior in a neutral or negatively pressurised vessel. The hole does not reseal because the septum is made of a material that can be 'cored out' by the needle after one or multiple insertions. Because this material is stretched in older designs, the hole tends to get larger.

## PROBLEMS SOLVED

The Safe-Septum solves these two particular problems. The Safe-Septum is available in an EPDM or silicone material, both of which do not easily core. The Safe-Septum cartridge is installed in a compressed state so that when a needle is removed from it, the sides of the penetration hole match perfectly for easier sealing and they are pressed together because of the compression of the originally installed material. Thus, the sides are forced together into a tight seal. These seals hold up under pressures of up to 30 psi, according to the testers of the Safe-Septum. After repeated needle penetration through the multiple, single-track needle ports in each Safe-Septum cartridge, no visible leakage occurs. Added insurance of the reduced risk of contamination comes from the unique, patented single-track QMI design that not only guides needles into yet-to-be-penetrated material, but allows the technician to see where he/she has pierced the material previously and, therefore, select another spot for insertion.

## MINOR CHANGES CAN LEAD TO MAJOR IMPROVEMENTS

As for many other industries, the pharmaceutical bioprocessing business is experiencing shrinking resources and increasingly strict quality requirements. Technicians must re-examine how they've done things in the past and make minor changes that lead to major improvements in how efficiently and cost effectively they do their jobs. Learning about the latest products is the first step, and integrating those products is the next step towards higher quality and lower costs.