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Managing Quality Through Use of Aseptic Transfer and Inoculation Systems

By Rulon A. Chappell, Ph.D.

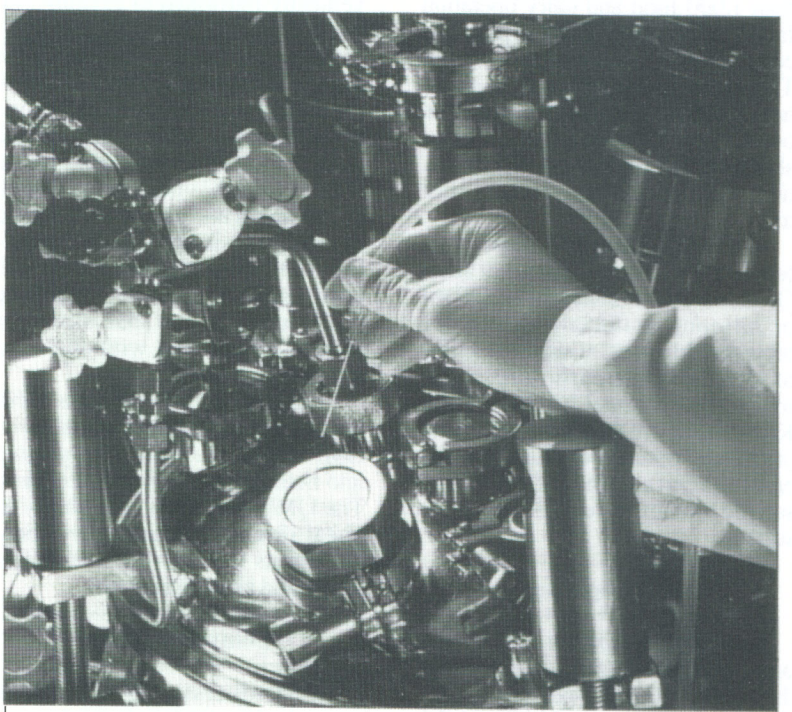
The generation of high-quality products is a primary objective for any research, biotechnology or pharmaceutical operation. In order to achieve consistent and documentable product safety and quality, goals must be clearly defined. Quality must be defined as: "conformance to specifications." Both product and process specifications must be developed to reflect product safety and process economy.

Product specifications should include:

- No measurable contamination
- Proper product composition and consistency
- Purity
- Desired physical attributes such as color and texture
- Safety

Process specifications should include:

- Identification of critical control points, (temperature, time, pH)
- Down-time controls
- Control of waste or rework
- Timely completion of projects
- Reduction or elimination of other quality losses, such as recalls and poor consumer perception
- Achievement of profit projections



The QMI Safe Septum features either seven or 12 ports, or guiding channels, for the needle. Research bioreactors will accommodate the seven-port, while most production bioreactors should be fitted with the 12-port.

applications arena

Proper process control is essential to attaining conformance to product and process specifications. In any laboratory, pilot plant or production plant operation, sampling, inoculation and addition of pH adjusting agents, nutrients or other materials to a closed bioreactor are necessary operations. The challenge for the bioreactor operator is to accomplish these functions without contaminating the process system. Maintaining pure culture is essential to any effective fermentation resulting in conformance to specifications, hence the production of safe products and the meeting of project objectives.

Table 1.

Experiment: Safe-Septum vs. Exposed Vessel and Control

Vessel	TA	pH	Host* CFU/ml	Phage** PFU/ml	E. coli CFU/ml
Control	0.16-0.19	6.4	10^6-10^7	0	0
Open	0.16-0.19	6.4	10^6-10^7	1-8	38-65
Closed	0.16-0.19	6.4	10^6-10^7	0	0

*colony-forming units **plaque-forming units

Aseptic Transfer

A proven source of bioreactor contamination and product and process failure has been the use of improperly designed and malfunctioning septums. **QMI** (St. Paul, MN) has manufactured and marketed aseptic transfer devices, or septums, specifically for the food and dairy industry for many years. These products have allowed aseptic access to processing lines, fermentation tanks and stainless steel storage vessels without the contamination of products or samples. These aseptic sampling and transfer systems are currently used in more than 1,000 food and dairy operation facilities and now several pharmaceutical and bioengineering facilities. They have proven effective in eliminating the contamination hazard that occurs during inoculation, troubleshooting for sources of contamination, and documenting process control.

A correctly-designed aseptic septum, such as the Safe-Septum from QMI, which is available in an EPDM or silicone material, allows the biotechnologist or research scientist to identify where each sampling or inoculation channel has been used. Knowing where the septum has been previously punctured allows the scientist to insert a fresh needle for a new sample into an unmarred area of the septum.

Safe-Septums

Safe-Septums feature either seven or 12 ports, or guiding channels, for the needle. The seven-port septum has an outside diameter of 1.5" and the 12-port septum has a diameter of 2.5". Most research bioreactors will accommodate the seven-port option, while most production bioreactors should be fitted with the 12-port septum, which will allow for use of a larger bore needle.

When selecting a septum, make sure it has been tested at extreme pressure, temperatures and time combinations. As a benchmark, the QMI septum has been tested at up to 280°F at 150 psi for 300 hours. To show that the septums are truly safe, they must also have been punctured with needles during this test time to insure that no leakage occurs when the needles are withdrawn.

There are several installation options when incorporating a septum into a bioreactor. The septum can be threaded into the top of the bioreactor, or it can be permanently welded in either insulated or non-insulated sidewalls of bioreactors. A septum can also be fitted onto a tri-clamp end cap and clamped onto an end cap port. The thread or tri-clamp connection option allows the septum to be conveniently retrofitted to existing systems.

Other important characteristics of a truly "safe" septum are: individual packaging and pre-sterilization via ethylene oxide—features that will add to the reduction of contamination risk. If the septum has been tested at high temperatures and pressures, it should be sterilizable in place in the bioreactor. The best designed septum will have a smooth surface on the outside of the injection port that allows for effective sanitizing with chemical sanitizing agents.

The use of "safe septums" will help bioreactor operators comply with GMP and can be easily incorporated into Standard Operating Procedures. The QMI septum has the ability to reseal after being punctured by a needle and its interior surface remains sterile and clean during use.

Controlled Study

The University of Minnesota researched the QMI Safe-Septum for safety and effectiveness before it was introduced to the biopharmaceutical industry. During the study, two bioreactors were exposed under a laboratory hood to an aerosol containing bacteriophage and *E. coli*. One vessel was pre-inoculated with a host organism and allowed to remain under the hood with an inoculation port open for two minutes. The second vessel remained closed and was inoculated with the host organism through the QMI Safe-Septum, using a syringe and needle. A control vessel was inoculated in the same manner and maintained outside the hood to avoid exposure to the aerosol.

Thus, the experiment consisted of a control outside the hood, an exposed vessel under the hood, and a vessel with the Safe-Septum in the hood. The aerosol contained bacteriophage specific to the host at 10^4-10^5 PFU/ml and *E. coli* at 10^6 CFU/ml in a liquid medium containing cottage cheese whey, milk and sweet whey solids. This solution was then propelled by pressurized freon into the laboratory hood to generate the aerosol. Three trials were conducted. The vessels were incubated at 22°C for 16 hours.

As *Table 1* shows, the host was able to grow and phage and *E. coli* were not detected in the control and closed system. However, in the open system, both *E. coli* and bacteriophage were detected. During this challenge study, the septum proved able to maintain control and avoid contamination of both bacteriophage and *E. coli*.

Reduce Contamination

To maximize profits, quality must be defined as conformance to specification. Specification for product and processing must include control of contamination. Safe septums are a proven method of reducing the contamination that can occur when ineffective septums or open ports are used for inoculation, sampling or addition of nutrients or other materials. The QMI Safe-Septum, with its ease of installation and user-friendly design, can help any research laboratory, pilot plant or production facility reduce the threat of contamination and improve product compliance with quality/safety specifications, while reducing research time and the frustration that occurs when bioreactors become contaminated.

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