APPLICATION NOTE

Risk Free Connection of Sterilized Single-Use Fluid Path Assemblies to Stainless Steel SIP Systems with Lynx® ST (Steam-To) Connectors

Table of Contents

Introduction

Disposable manufacturing, the replacement of reusable components in a manufacturing operation with single use components, is beginning to have a significant impact on manufacturing. This trend is dynamic and has moved from single use capsule filters to the integration of single use filtration and containment systems with tubing and a range of different connection components to make up disposable fluid path assemblies. These assemblies, illustrated in Figure 1, are then gamma sterilized at > 25kGy and delivered sterile packed and ready for use.

Integration of disposable manufacturing products with stainless steel process components has resulted in industry-wide economic and security benefits. Cost savings stem from reduced labor and validation efforts, greater speed to market for clinical materials, and smaller, more flexible manufacturing facilities. Single use components eliminate the need to clean in place (CIP) and significantly reduce the risk of product contamination from other drugs or from CIP solution. Also, disposable fluid paths help remove the operators from the vicinity of critical operations, ensuring enhanced process security.

Traditional stainless steel manufacturing process, particularly in aseptic processing, typically relies on steam sterilization, steam in place (SIP) or autoclave to sterilize the process components, housings and connections. In many situations steam sterilization of the entire system is impossible and aseptic connections have to be made in a Class C or B environment by highly trained, appropriately gowned operators. The process is complex and can be unreliable. The 2001 PDA survey on aseptic processing[1] identified aseptic connections as one of the more common reasons for aseptic processing sterility failure. Usually, aseptic connections are made using two Tri-Clover® connectors. First,the connectors must be covered and sterilized. Once sterilization is complete, removing the sterile covering from each connector then rapidly joining and tightening the union completes the aseptic connection.

The integrity of aseptic connection relies on the cleanliness of the area in which the connection is being made, the competence of the operator and a little luck. The execution of consistently reliable aseptic connections requires detailed SOPs and careful, regular operator training[2]. Even then thereare no guarantees.

MILLIPORE

Lynx ST Connector Eliminates Need for Aseptic Connections

The Lynx ST connector is a single use gamma sterilizable connector designed to connect pre-assembled, pre-sterilized filters, tubing, sample bags and flexible container systems to permanent sterile processing systems. The Lynx ST family of connectors is available with the connection options as described in Table 1.

The outlet side of the Lynx ST connector is connected to a disposable fluid path as illustrated in Figures 1 and 2. The Lynx ST connector facilitates the gamma sterility of disposable fluid flow paths by gamma radiation. The closed seal on the Tri-Clover end of the connector assures that the flow path remains sterile prior to face steaming once connected to process piping or product tanks.

Making the fluid path connection to the stainless steel components is easier than ever. The Tri-Clover end of the Lynx ST connector is simply connected directly to the stainless steel component to be steamed in place. As the exposed face of the connector will be steam sterilized along with the rest of the stainless steel feed system, aseptic connection practices are not required. In this way secure sterile connections between stainless steel and disposable fluid paths can be made in Class B, C or D environments by minimally trained operators using simple SOPs.

The closed face of the connector not only prevents contamination from entering the flow path but also protects the flow path from thermal damage and condensation build up during the SIP cycle. The sterilizability of the Lynx ST connector fluid path by gamma irradiation and SIP sterilization of the Tri-Clover interface have been demonstrated and recorded[3].

Inlet	Outlet	Part Number
¾ in. TC Flange	¼ in. Hose Barb	STC11FHA01
1 ½ in. TC Flange	½ in. Hose Barb	STC21THN01
1 ½ in. TC Flange	1 in. Hose Barb	STC31THN01
1 ½ in. TC Flange	1 ½ in. TC Flange	STC4ITTN01

Table 1. *Connection options for the Lynx ST family of connectors.*

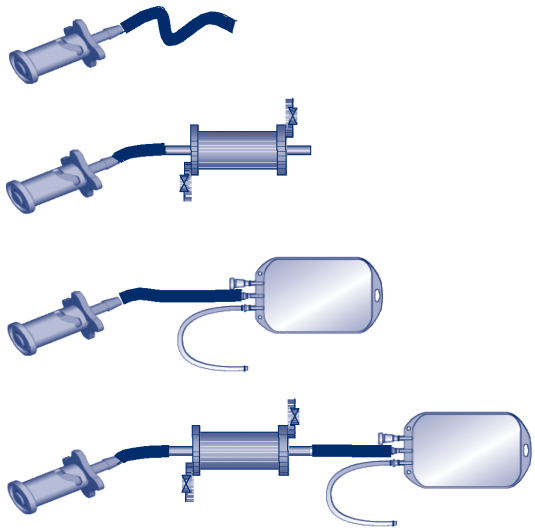

Figure 1. *Examples of disposable fluid path assemblies.*

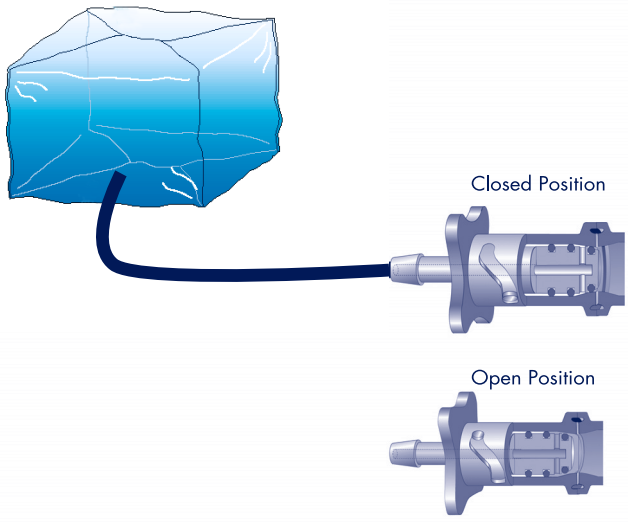

Figure 2. *Lynx ST connector connected to a fluid path, showing both open and closed positions.*

Making and Using Secure Sterile Connections with the Lynx ST Connector

The process of connecting to stainless steel tanks, piping, bioreactors, and other fixed process equipment is straightforward and secure. The detailed method of use may vary depending on individual circumstances. First, as a general rule to avoid contamination of successively cleaner areas, the fluid path is removed from its final packaging close to where it will be installed. The Lynx ST connector with a Tri-Clover fitting is mated to the Tri-Clover fitting component of the stainless steel system and securely tightened. The stainless steel Tri-Clover connection arrangement must employ good SIP practices to ensure the removal of condensate and air, thus allowing the face of the Lynx ST connector to reach the target temperature between 121 °C and 135 °C[4]. Once the SIP and cooling cycles are complete, the fluid path is ready for use. Simply twisting the Lynx ST cam-based actuation mechanism allows liquid to pass into the fluid path as shown in Figure 3.

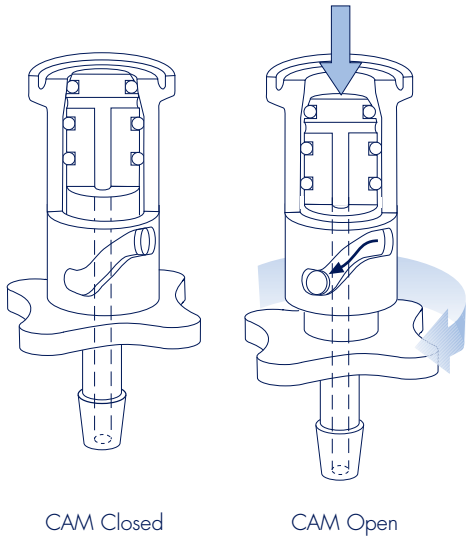

Figure 3. *Open position of Lynx ST connector, allowing liquid to pass into fluid path.*

Applications

The Lynx ST connector is suitable for a broad range of applications including both sterile and non-sterile liquid transfer as well as static microbiological and biochemical sampling.

Sterile Liquid Transfer

Figure 4 illustrates a typical sterile dynamic flow-through fluid path; specifically, a final fill in which a disposable single use fluid path delivers sterile drug liquid from a stainless steel sterile holding tank to a filling machine. In this typical installation, the stainless steel holding tank (A) can be either sterilized in a "walk in autoclave" or SIP'd. In the case of SIP, the tank, often in a Class C environment, is steam sterilized along with a sterilizing grade liquid filter on the inlet and a sterilizing grade hydrophobic filter on the vent up to a closed Tri-Clover outlet. The drug solution is then sterile filtered into the holding tank. The filling machine is SIP'd in-situ

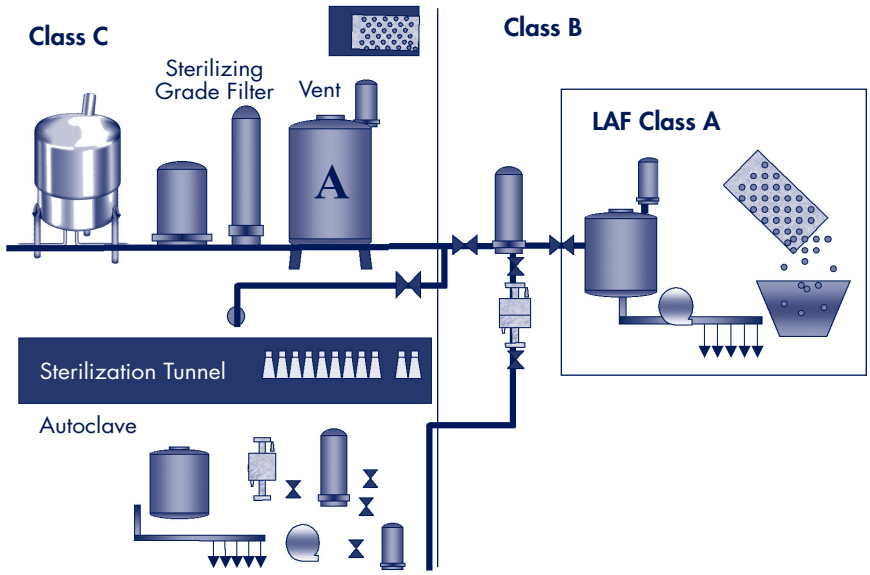

Figure 4. *Typical sterile dynamic flow-through fluid path.*

or aseptically assembled in its Class A environment. The sterile fluid path, usually silicone tubing with a sterilizing filter attached, is autoclave sterilized. One end of the sterile fluid path is connected to the filling machine inside the Class A environment. The other end is attached to the sterile holding tank by way of an aseptic connection, as shown in Figure 5.

The aseptic connection requires the careful removal of the stainless steel Tri-Clover closure from the holding tank, removal of the kraft paper covering the inlet of the sterilized fluid path, and the rapid connection of the two sterilized Tri-Clover unions. The integrity of the connection is dependent upon the skill and speed of the operator.

In contrast, using the Lynx ST connector makes the sterility of the Tri-Clover connection much more secure. As shown in Figure 6, the inclusion of a closed Lynx ST connector on the inlet of the gamma pre-sterilized fluid path assembly will facilitate a sterile connection. In this scenario, the pre-sterile fluid path is connected to the holding tank, and the holding tank is SIP'd or autoclaved with the appropriate sterilizing grade filters up to the Tri-Clover connection. In this way, the connection interface between the tank and the plastic fluid path is steam sterilized. No aseptic connection is required.

The Lynx ST connector, in conjunction with single use flow through fluid paths, is adaptable to almost any combination of single use components, including flexible tubing, connectors, manifolds, filter capsules, bags, etc. Sterilization options will need to be matched considering the materials of construction of each element of the disposable fluid path.

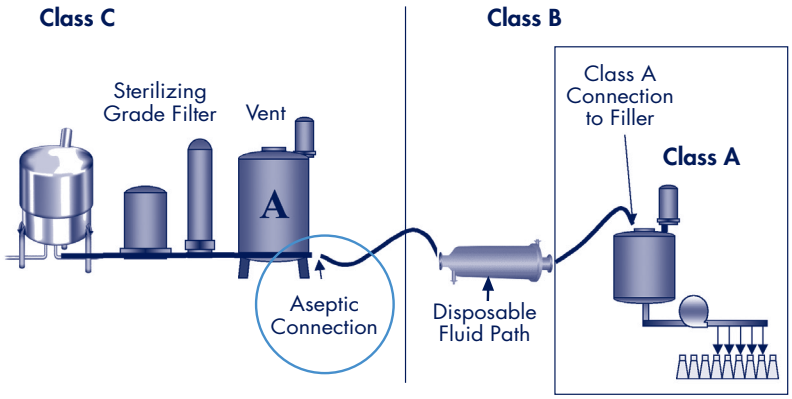

Figure 5. Aseptic connection *attaching sterile fluid path to sterile holding tank.*

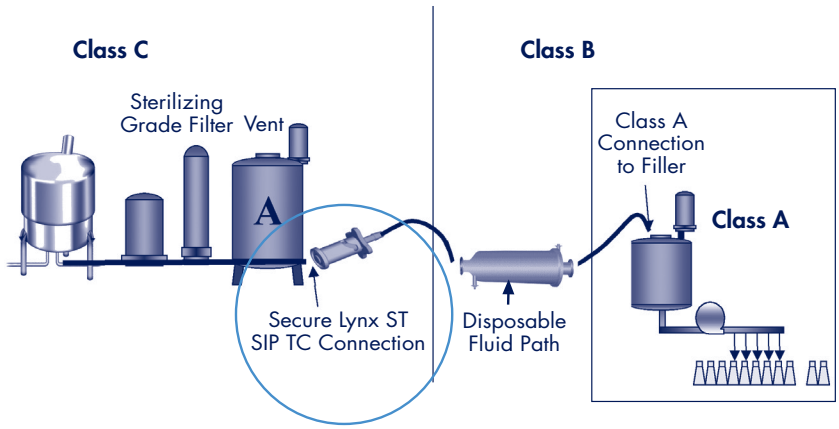

Figure 6. Lynx ST connector *eliminates need for aseptic connection for fluid path in Class C area.*

Another example of a sterile dynamic flow-through fluid path is illustrated in Figure 7. In this example, disposable manufacturing has been applied to the complete process; so risky aseptic connections are replaced with secure sterile connections with all SIP processes managed in a Class C environment. Large complex pre-sterilized fluid paths are delivered in easy to handle assemblies. Figure 7 shows a pre-sterilized, single use disposable fluid path delivering sterile drug liquid from a disposable flexible sterile holding tank connected via an SIP transfer flow control plate to a filling machine. The fluid path has been split into two components: a flexible 200 L container complete with sterilizing grade filters, and a pre-sterilized filling feed complete with sterilizing grade filter and filling components. Both components are equipped with a Lynx ST connector and can be joined together using a SIP interface to ensure a secure and sterile union.

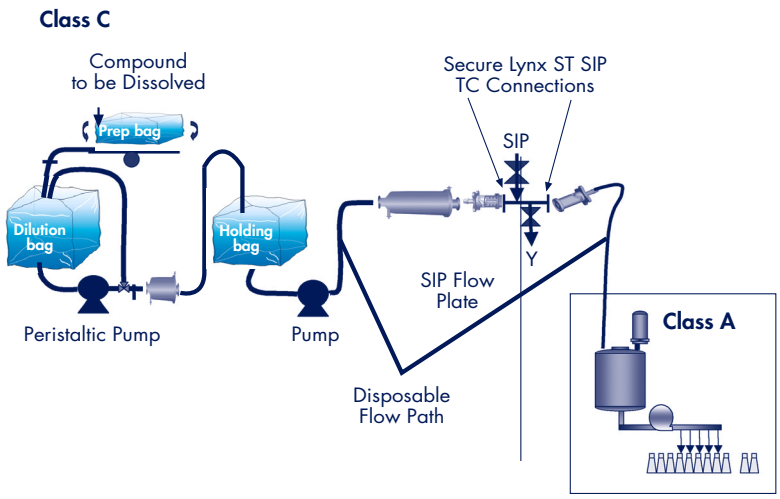

Figure 7. *Disposable manufacturing applied to sterile dynamic flow-through fluid path.*

Non-sterile Liquid Transfer

The use of the Lynx ST connector as part of a gamma pre-sterilized fluid path also provides a valuable option for some of the less critical downstream processes around harvesting, clarification, chromatography, and ultrafiltration. Many of these processes, although not truly sterile, are run as clean operations in an attempt to control bioburden. A packaged, pre-sterilized fluid path adapted with the Lynx ST connector can be integrated into a fully prepared downstream processing operation. Once the sterile fluid path is removed from its sterile packaging, the Tri-Clover fitting Lynx ST inlet is connected using a simple Tri-Clover/Tri-Clover union. The use of this technology when plastic to steel unions are required permits economical bioburden control.

Static Sampling

The requirement for direct process sampling is increasing. Whether the result of increased regulatory compliance activity or internal quality policies, more samples are being taken for more reasons more regularly. As a part of the process of pharmaceutical manufacturing, the exact location and number of sampling points required are determined by the process. The nature of the process step determines if a single sample is needed at a single point in time or if, as in the case of long duration processes, multiple samples over a period of time are needed. The samples generated by these procedures undergo two general categories of testing: biochemical analysis and microbiological analysis. Although the micro-biological integrity of the sample is obviously more critical for micro-biological analysis than it is for biochemical analysis, the micro-biological integrity of the process is usually critical and should not be compromised in any way by the sampling procedure. This is important in both sterile and non-sterile systems.

The purpose of microbiological analysis of a process sample is to determine either the sterility of, or bioburden level present in that sample. Should that sample be contaminated by a poor sampling procedure, the resulting "false positive" would drive a series of unnecessary Out of Specification (OOS) activities that at best could cause costly inconvenience and at worst could lead to the loss of the batch. Equally, if as a result of poor sampling technique an otherwise perfectly sterile process step is contaminated, then all the precautions taken during the manufacturing process are wasted and the fundamental purpose of sample monitoring is negated. Imagine the consequences of contaminating a bioreactor in week 1 of a 3-week culture or of contaminating a final sterile product holding tank.

The Lynx ST connector can play a key role in avoiding contamination during static sampling applications. The advent of disposable manufacturing offers some significant benefits, notably in the use of "closed" sampling systems. The example highlighted in Figure 8 demonstrates sampling of a bioreactor for both microbiological and biochemical analysis, but the principles can be equally well applied to all sampling applications.

Consider a 2000 L bioreactor that is typically sampled three times during its culture cycle. The sampling requirements are for three 500 mL samples for microbiological analysis and 3 x 50 mL samples for biochemical analysis. Traditionally, the samples are taken sequentially using small flexible containers arranged on a single use pre-sterilized fluid path. Although this sample system is delivered gamma pre-sterilized, it must be assembled to an autoclave sterilized valve arrangement inside a laminar flow hood. Then, the aseptic valve assembly is fitted to the bioreactor and the reactor and sample valve interface is steam sterilized. The problems of this particular procedure are as follows:

- Inconvenient and labor intensive
- Risk associated with the aseptic assembly
- Significant capital cost of valves associated with the need for multiple sample points from multiple process reactors.

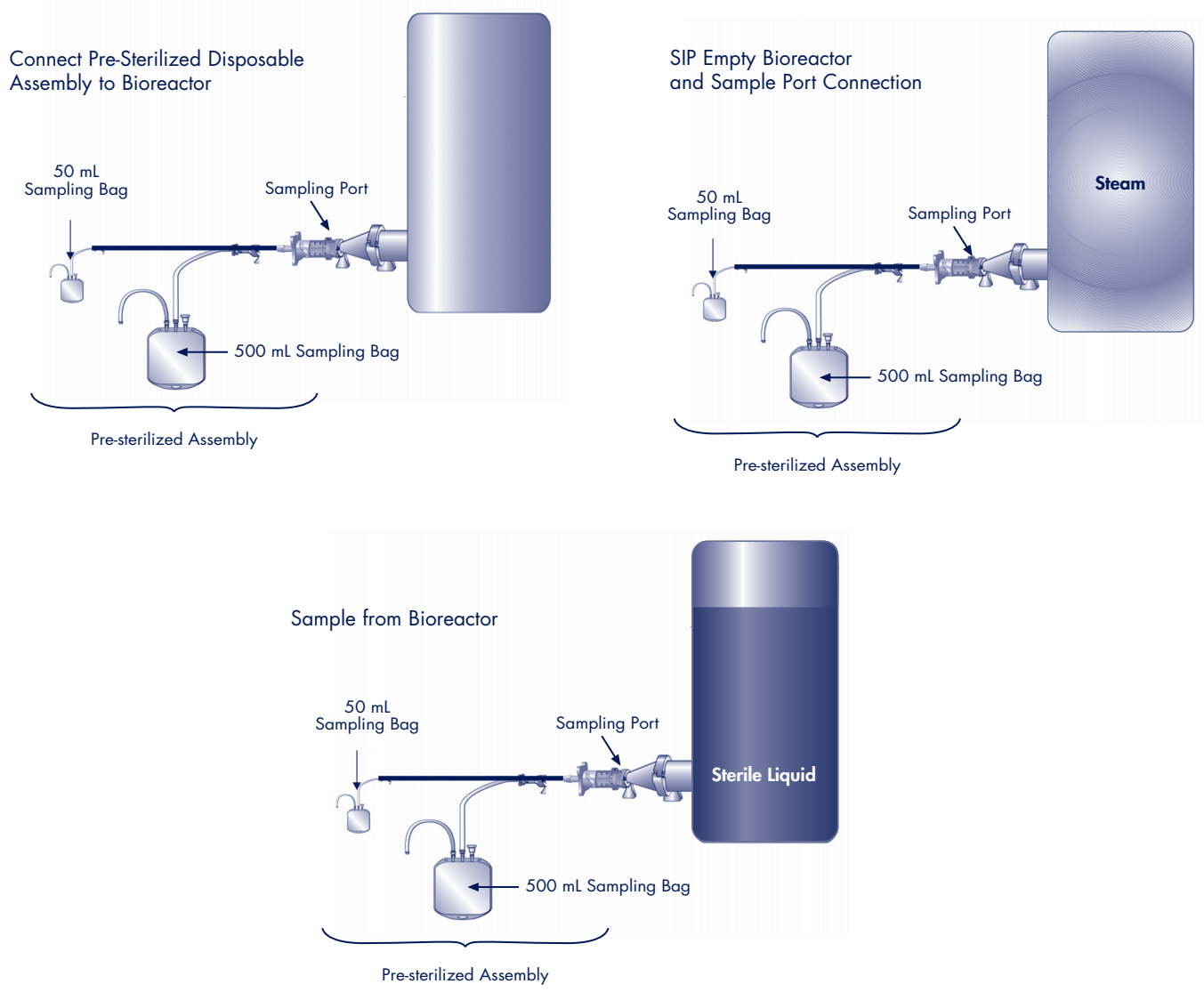

Figure 8. *Biochemical and microbiological sampling of a bioreactor.*

The Lynx ST connector can provide a solution to the problems associated with the above procedure. By fitting a Lynx ST connector to the existing pre-sterilized single use sample system as shown in Figure 8, the system can be attached directly to the bioreactor with the connection interface being sterilized by a SIP process in exactly the same way as the stainless steel valve. The Lynx ST connector's unique double internal seal design prevents the risk of contamination through the body of the valve during actuation and makes it an optimal choice for this application.

Conclusions

The Lynx ST connector valve is a valuable component of single use, disposable fluid paths. They are used in conjunction with SIP processes to replace aseptic connections for the secure connection of pre-assembled, pre-sterilized single use fluid paths to stainless steel systems or to each other.

The patented secure actuation control enables the use of Lynx ST technology in both dynamic flow-through applications as well as static sampling applications.

References

1. PDA Technical Note No. 36 *Current Practices in the Validation of Aseptic Processing –2001.* May–June 2002.
2. *Proper Gowning for Cleaning and Use of a Lamina Flow Hood, Including Aseptic Techniques Guidelines.* Millipore SOP, No. 00634 6250, Rev. B.
3. *Lynx ST Validation Guide,* Millipore Corporation Validation Guide, 2003; Lit. No. VG2358EN00, Rev. – .
4. *Guidelines for Using the Lynx ST Connector*, Millipore Corporation Technical Brief, 2003; Lit. No. TB2137EN00, Rev. – .

Discover the More in Millipore™

In every application, every step and every scale, count on Millipore to be everywhere for you—from monoclonals to vaccines, from clinical through pilot to full-scale manufacturing. Our technologies are used by most of the world's major biopharmaceutical companies. But we deliver more than advanced separation, purification, sterilization and quality control products. With Millipore, you get services to optimize and validate your processes, comprehensive resources to streamline and enhance your operation, unmatched know how forged from nearly 50 years' experience—and solutions that integrate it all. For higher yields, improved process economics and faster speed to market, discover the more in Millipore.

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:
In the U.S. and Canada, call toll-free 1-800-MILLIPORE (1-800-645-5476)
In the U.S., Canada and Puerto Rico, fax orders to
1-800-MILLIFX (1-800-645-5439)
Outside of North America contact your local office.
To find the office nearest you visit www.millipore.com/offices.
Internet: www.millipore.com
Technical Service: www.millipore.com/techservice

MILLIPORE

Millipore and Lynx are registered trademarks of Millipore Corporation.
"Discover the More in Millipore" is a trademark of Millipore Corporation.
Tri-Clover is a registered trademark of Alfa Laval.
Lit. No. AN7428EN00 Rev. A 5/08 BP-GEN-08-00240 Printed in the U.S.A.
© 2008 Millipore Corporation, Billerica, MA U.S.A. All rights reserved.