
CPC
A DOVER COMPANY
THE PATH TO CONFIDENCE
AT EVERY POINT OF CONNECTION

Introduction

CONFIDENCE AT EVERY POINT OF CONNECTION: VALIDATION AND TESTING METHODS USED IN SINGLE-USE TECHNOLOGIES

Single-use technology (SUT) has transformed biopharmaceutical development and manufacturing. SUT bioreactors reduce manufacturing costs and offer streamlined production for small batches — a growing trend that makes targeted drug development for specific patient populations possible.

While the benefits of SUT in bioprocessing are clear, the pathways to successfully applying SUT continue to rapidly evolve. Today's bioprocessors manage quick turnaround times, simultaneous processing of multiple batches, and flexible manufacturing setups — all of which require highly reliable, easy-to-implement, and intuitive solutions.

The ability to seamlessly combine multiple components and systems is critical. Process containers, tubing manifolds, transfer lines, mixing and storage tanks, chromatography skids, capsule bioreactors, and other bioprocess equipment must be securely brought together to maintain system integrity and safeguard drug quality.

Robust validation is at the core of optimal SUT application in bioprocessing. Every point of connection is a potential point of vulnerability. Bioprocessors must have complete confidence that all components are rigorously tested, thoroughly validated, and function as intended.

In this publication, CPC shares its expertise in SUT validation formed from more than 25 years in the bioprocessing industry. You will find practical information on key issues including extractable/leachable testing in components, freeze/thaw in single-use assemblies, efficiencies driven by SUT advances like genderless connectors, and more.

As a global leader in single-use connection technology, CPC's well-tested, robust, simple-to-operate solutions help reduce system complexity, production expenses, and risks. With deep bioprocessing experience and an unmatched range of sterile and genderless connectors in its AseptiQuik® line, CPC partners with bioprocessors worldwide to achieve reliability, quality, and efficiency in biomanufacturing — today and tomorrow.

North America

St. Paul - Corporate Headquarters

1001 Westgate Drive

St. Paul, MN 55114

Toll Free: +1 800 519-7633

Phone: 651-645-0091

Europe

Mörfelden-Walldorf, Germany

Kurhessenstrasse 15

64546 Mörfelden-Walldorf

Telefon: +49-6026-9973-0

Asia Pacific

Hong Kong S.A.R. of China

Flat B, 29/F, West Gate Tower,
7 Wing Hong Street,

Cheung Sha Wan.

Kowloon,

Hong Kong S.A.R. of China

Phone: (852) 2987-5272

China

Shanghai

Room 1802, Building A, IBC,

391 Guiping Road, Xuhui District,

Shanghai, China 200233

Phone: +86 21 2411 2666

Toll free: +86 400 990 1978

Contents

How to Overcome Validation Challenges In A Single-Use World
By: Derek Pendlebury
4

Single-Use Systems for Storing and Shipping Frozen Drug Materials – Every Component Counts
By: Derek Pendlebury
10

Extractables Testing On Single-Use Connectors
By: Ele Vesel
14

The Path To Genderless Connectors: How Genderless Sterile Connectors Lead To More Flexibility, Faster Changeovers And Reduced Costs
By: Todd Andrews
17

DEREK PENDLEBURY is the global channel manager for bioprocessing at CPC and has worked for over 34 years in the development and supply of SUTs for the biopharmaceutical industry. His experiences include sales, product management and development, sales management, corporate marketing, and corporate management in senior positions with Sartorius, Pall Corporation, Agilent Technologies, 3M, ATMI, and Charter Medical. Dr. Pendlebury has authored numerous papers and book chapters and has presented on SUTs at over 20 conferences. He is an active member with the BPSA, PDA, and ISPE.

ELE VESEL is the senior quality engineer for bioprocessing at CPC and has experience in quality, auditing, and clean room manufacturing of medical devices for over 16 years. She also holds a black belt in lean manufacturing from the Institute of Industrial Engineers. Vesel is an active member of ASQ and the Henrici Society for Microbiologists.

TODD ANDREWS is the global sales and business development manager for bioprocessing at CPC and has worked for over 16 years with a focus on single-use technology development and supply for the bioprocessing industry. His experiences include sales, business development, and product management. Andrews is an active member with the BPSA.

How to Overcome
Validation Challenges In A Single-Use World

By
Derek Pendlebury
OEM Channel
Manager Bioprocessing
CPC - Colder Products Company

Single-use systems (SUS) are changing the way end users think about validation. The complex supply chains of SUS are not always as robust as necessary. This presents both manufacturers and end users of SUS with validation challenges not present with a stainless steel equivalent. Given increased regulatory scrutiny on supply chain security and risk mitigation strategies throughout the development and manufacture of a therapeutic drug product, how can end users ensure the expected level of compliance in this new world of SUS? The secret: shared responsibility for validation with your supply chain.

WHY VALIDATION NEEDS TO BE A SHARED RESPONSIBILITY

Therapeutic drug manufacturers today face multiple challenges to produce safe and effective drugs. These include: downward cost pressures, in a highly regulated market, with a multitiered supply chain. The trend away from stainless steel-based processes to single-use processes introduces a myriad of different suppliers and points of failure. Starting at the component level, suppliers use multiple raw materials in their supply chain. Some of the raw materials needed to manufacture the parts they supply to system integrators are themselves individual components with their own raw material supply chain. Many system integrators not only fabricate systems, but also manufacture some of the components used in a single-use assembly. Therefore, the system integrators also have their own raw material supply chain to manage and validate before they assemble and supply the finished system. Validation of fixed pipe-based manufacturing systems used to be the primary responsibility of the drug manufacturer. However, that model is changing. It is not rigorous enough to ensure reliable and repeatable performance of all the products delivered from all suppliers of an SUS. In this new SUS world, validation needs to begin at the component raw material level and continue successively through all manufacturing, operational, and supply steps to the final assembly. Many drug manufacturers understand how to validate in their own environment. But what does a rigorous validation program look like for their component suppliers and systems integrators?

THE CHALLENGES OF COMPONENT VALIDATION

The basic building blocks of a single-use assembly are the components. Common components include connectors, filters, tubing, clamps, cable ties, ports, and bag chambers. This is where validation begins for the completed SUS. The drug manufacturer and system integrator need to ensure quality controls and robust systems are in place. Yet, several challenges exist:

- *Lack of a standard approach* — One of the challenges for component validation is the lack of applicable standards or uniformity in the industry. This leads to several issues:
  - lack of a reliable and repeatable production process
  - inability to measure quality and performance accurately

- inefficiency caused by training required for different components
- inconsistencies across facilities
- restriction of implementation of flexibility due to limited interoperability

Several industry groups such as BPSA, BPE, and BPOG are proposing uniform procedures and methods, but major challenges still remain. For example, plastic films used in the manufacture of single-use bags are currently regulated under USP<661>, which is a standard written specifically for packaging. A proposed new standard (USP<665>) specifically for polymer components and systems used in manufacturing pharmaceutical and biopharmaceutical drug products is currently out for comment, but it is not yet an industry standard. However, as specific standards for single-use technologies evolve, the component suppliers will start to converge.

- *Variability among suppliers* — Some suppliers have a specific focus on meeting the needs of the biopharmaceutical industry, while others enter the SUS supply chain from industries where different levels of control, documentation, validation, and cleanliness apply. Suppliers entering from other industries are challenged with limited in-house bioprocess expertise and often a lack understanding about the requirements of the system integrator, drug manufacturer, industry, and the regulatory bodies. Many suppliers are moving toward cleaner manufacturing and assembly processes, including clean room manufacturing (typically ISO Class 7), clean component molding, and clean extrusion capabilities.
- *Understanding end users' needs* — Component suppliers can be many manufacturing steps away from the drug manufacturer who is the ultimate end user of the SUS. The resulting lack of direct communication with the user makes understanding their needs a challenge. In addition, component suppliers have to not only meet the needs of end users, but also the needs of their system integrator customers. This may impose additional requirements on the component supplier. Examples of this include: batch records, quality documentation, lot traceability, and return goods processes required to support the integrators' manufacturing and supply chain specifications.

Despite these challenges, as the market matures, so do the players and their approaches to validation. The end users' expectation is a robust, scientific approach that results in a stable and dimensionally centered process.

BEST PRACTICES FOR COMPONENT VALIDATION

What can component manufacturers do to produce and validate their products to help integrators and GMP manufacturers meet their regulatory needs? Component suppliers can do this primarily through validation of the product supply and design and validation of the manufacturing process.

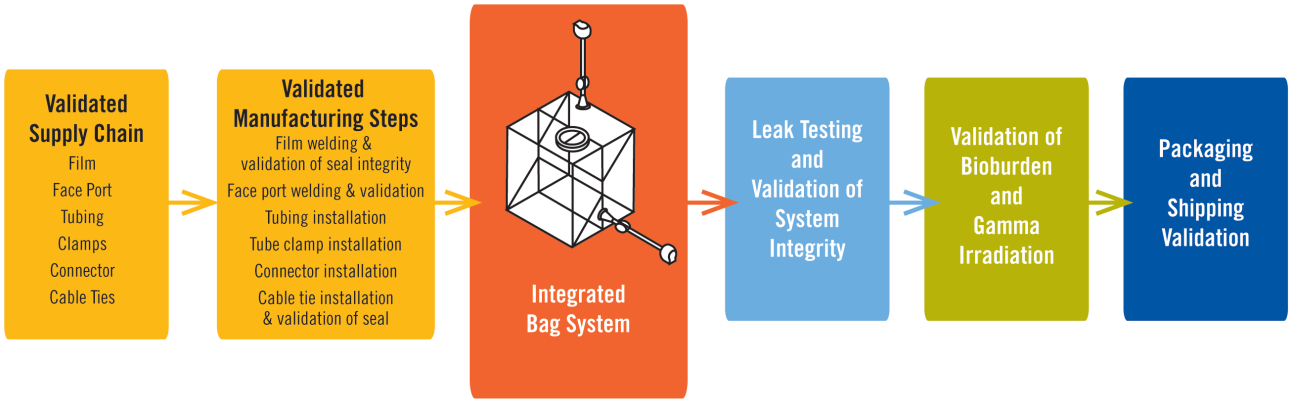
Validation of the product supply and design.

- *Material and Supplier qualification* — A robust approach starts with both raw material and supplier qualification. Ensuring raw materials meet the standards required for the finished component can be a lengthy process. Typically, most companies have industry-acceptable materials identified, and they select one of these core materials of construction that meets the performance requirements. Qualifying material suppliers up front is equally important. Some important criteria include: the ability to meet both current and future anticipated requirements, expertise and control to manufacture and supply a consistent product, and financial health.
- *Rigorous design process* — After identifying the appropriate materials of construction and defining the component of design, the supplier should perform a robust manufacturing validation process. This includes functional testing, mold validations, qualification of assembly of the component (if required), physical and chemical testing to specifications, dimensional tolerances, and establishing specification ranges. Elements include installation qualifications, operations qualifications, and process qualifications (IQ, OQ, PQ), which focus on the equipment, the critical process parameters, and the stability of those processes.

Validation of the manufacturing process.

- *Assembly process validation (if required)* — IQ, OQ, and PQ are undertaken to ensure that good product with reproducible performance is manufac-

IQ	**Installation Qualification**	Verify equipment design features and ensure correct installation and calibration
OQ	**Operation Qualification**	Establish control limits for a process that produces product to meet predetermined requirements
PQ	**Performance Qualification**	Demonstrate the process consistently produces product that meets all predetermined requirements under normal operating conditions



Manufacturing validation of a simple 3-dimensional bag assembly

tured across the complete specification range, including the steps required to assemble the finished product.

- *Product performance validation* — The final product is validated to ensure it meets operational specifications. The PQ is performed on the nominal, the optimized position relative to the settings of a process, for an extended period of time depending on the components. Up to three lots are made during PQ to collect additional data points to confirm a controlled and consistent process. During this time, validation, technical, installation, and application documents are developed, extractables testing is performed, and biocompatibility and reliability data are developed.
- *Inspection* — One way to ensure that a product is fit for the role it is designed for is to undertake 100 percent inspection and/or testing prior to final packaging and shipping. A successful final test not only proves the product is fit for purpose, but also acts to validate that the manufacturing process is producing good product. A visual inspection will detect gross flaws but may not detect smaller flaws that could result in product failure. It may not always be feasible, or even possible, to undertake 100 percent product testing. If the test method is destructive, or could result in potential contamination or damage to the product, then it is unsuitable as a final test. The increased focus on SUS performance and validation, coupled with recent advances in ease of use and accuracy, has allowed noninvasive test methods such as a 100 percent helium leak test to be easily integrated into the final testing of certain components.

An evolving requirement is the expectation that the quality systems mirror that of a GMP operation. For component suppliers, minimum expectations include:

- Compliant formalized system
- Written quality manual
- Full product traceability
- Manufacturing controls
- Ability to handle formal customer quality audits

While these are not GMP-regulated, many component manufacturers describe their goal as being "GMPcompliant."

Drug manufacturers are now auditing component suppliers to the same standards and with the same expectations as full system integrators. This approach serves to allow the drug manufacturers to both understand the whole supply chain for SUS and to drive the quality and validation requirements throughout the complete sourcing and manufacturing process.

THE CHALLENGES OF SYSTEMS INTEGRATOR VALIDATION

What does it take to supply a complete single-use sterile system to the biopharma market? It can be a complex process — even for a relatively simple product such as a storage bag comprised only of flexible film, face ports, tubing, clamps, connectors, and cable ties, double bagged and gamma irradiated.

Most integrators do not manufacture all of the products used in a single-use assembly. Some integrators don't manufacture any of the components. This complex supply chain can include both external and internal suppliers, and in some cases, all suppliers will be external. The number of components, assembly steps, and the actual suppliers may vary depending on the design and complexity of the final assembly.

BEST PRACTICES FOR SYSTEMS INTEGRATORS VALIDATION

Key requirements for achieving product quality at the systems integrator level include the following:

Manage the Supplier Base

Final product quality starts with selection, qualification, and validation of all raw material and

component suppliers. This evaluation is the same as that undertaken by component suppliers but is critical to ensure that both parties are comfortable working together. One framework for supplier evaluation is called the 10-Cs of supplier evaluation.

Once a supplier has been selected, it must be qualified and validated. That includes validating manufacturing quality across all processes the supplier has in place: the quality program, product certification, returns process, paper and site audits, risk mitigation strategy, their supply chain security program, manufacturing controls, raw materials sourcing strategy, and corrective action process. From a risk management standpoint, do they dual source? Do they make or outsource? Do they have an active continuous improvement process and a new product development program that can support the integrator's program?

Once a supplier is chosen, validation continues on the selected supplier's ongoing processes. This includes but is not limited to quality, form, fit, and function of parts, service, delivery, and supplier score cards.

Manufacturing Considerations

From a system manufacturing perspective, customization is one of the greatest advantages of single-use technology, but also a disadvantage. Customization presents challenges for supply chain management and product validation. Recognizing that different levels of validation are needed based on the level of customization, many manufacturers have adopted a multitier approach to system supply.

Manufacturing Validation

The focus on product quality continues through the validation of the manufacturing process, facility, equipment, and personnel who manufacture the assemblies.

- *Parts and raw materials* — Assess whether the component parts and raw materials meet the specifications required for quantity, cleanliness, documentation, and visual inspection, and conformance to the bill of materials (BOM) for the assembly to be built.
- *Assembly* — The assembly process requires multiple levels of validation to answer questions that include:
  - Are components that can only be used one way assembled in the right orientation?
  - Are the weld temperatures and the dwell times on the welding systems correct to ensure a reliable seal?
  - Does the seal strength meet specifications?
  - Are all parts documented?
  - Are the guns used to apply the cable ties validated for the correct torque?
  - Does the final assembly conform to the BOM?
  - Is there documentation that each of the in-process quality assurance tests are performed and passed?
- *Personnel* — Critical considerations here include documented training and certification of the assembly and manufacturing

SUPPLIER EVALUATION 10-Cs

ATTRIBUTE	EXPLANATION
Capacity	Does the supplier have adequate engine room to produce your goods? Capacity includes equipment, human resources, materials and space. Can your supplier adjust their capacity in line with your requirements?
Cash	Does your supplier have adequate financial standing and resources? This is especially important if you expect your business to grow.
Clean	Does your supplier have an appropriate sustainability policy?
Commitment	Quality is a key requirement for any business – does your supplier have the commitment to maintain suitable quality performance?
Communication	What tools will you utilize to communicate with your supplier? Another key point is who will communicate with who. For example consider how you will manage problem resolution and issue escalation.
Competency	Does your supplier have the skills to deliver the materials you require?
Consistency	Does your supplier guarantee and deliver a consistent product every time and are they on time with their deliveries?
Control	Is your supplier in control of their policies and procedures? Can they ensure that their performance can be consistent?
Cost	What is their cost of goods and do they have their own supply chain under control?
Culture	Does your supplier share the same cultural values as your organization? Does it make sense that your supplier share similar values and attributes to avoid strains in future relationships?

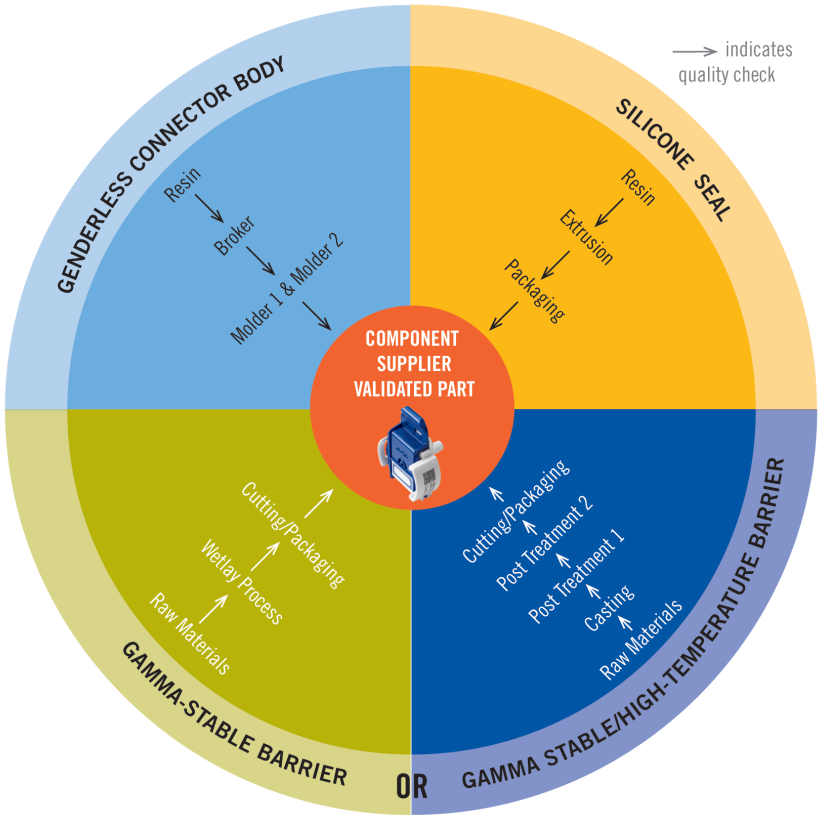
personnel (e.g., operations, gowning, hygiene, and inspections), internal and external audits of the manufacturing processes, regular retraining and refresher training, continuous improvement programs, and six-sigma continuous improvement processes.

- *Facility* — Important validation considerations for the facility include:
  - Operation and performance of the manufacturing environment to the required cleanliness standards
  - Robust preventative maintenance schedule for all manufacturing and ancillary equipment
  - Continual monitoring of critical process parameters such as particulates, bioburden, temperature, pressure, and humidity
  - Continual monitoring and validation of the set alarm levels that trigger alerts and actions.
- *Product* — Finally, the finished product is validated prior to sending to the end user. This validation is designed to ensure that all of the work previously undertaken on raw materials, components, and manufacturing results in a product that meets the requirements of the end user:
  - Is it integral, and fit for purpose?
  - Does it conform to the design specifications for the customer?
  - Is the packaging validated to protect the product during inventory, shipping, handling, and storage prior to use?
  - Is the irradiation process validated and certified?
  - Is the assembled product fully traceable through batch records to allow identification of components/processes in the event of a failure?
  - Does the product meet all appropriate industry standards?
  - Is it certified to the level that the end user customer wants it to be?

5 CONSIDERATIONS FOR THE DRUG MANUFACTURER

Industry collaboration is still needed. As with the adoption of any new technology, there will be challenges with single-use technology until it matures. In the meantime, there are some critical areas where drug manufacturers and suppliers can work together to make the adoption of single-use technology simpler, more efficient, and less daunting for the benefit of the industry as a whole.

1. One challenge for the drug manufacturer is setting clear expectations for the suppliers. Setting expectations requires a significant amount of communication with each supplier. This is where industry standards can be of great benefit. Industry groups such as ASME, BPE, BPSA, BPOG, and ASTM are the way to reach consensus and efficiency for product standards. Industry groups enable participants to come to agreements in a noncompetitive and nonconfrontational setting.
2. An unmet need in the industry today is the drug manufacturers' desire for suppliers to provide more information about the useable range for components in their validation guides. End users are seeking more information about the limits of both the components and the assembled systems. Communication between end users and



Manufacturing validation of a critical Single-Use System component

suppliers to identify limits, and developing a joint understanding of what's required and what's possible, will help define the operational parameters of single-use technologies.

3. Variation is a threat to validation. Validation needs to take into account that things may change, not just over time on a large scale, but maybe even from batch to batch or item to item. Trying to get a sense for the intrinsic variability of systems should be considered for validation guides. One way to do that is to increase process controls to reduce the variation of production processes, in which case validation at a point may be closer to validation in a population.
4. Change control procedures are needed. In addition, consensus is needed on what constitutes a change, and how that change should be communicated. Understanding what the original validation was, in the context of any changes that happen, helps determine what confirmatory or additional testing should be done.
5. Finally, with this great new SUS technology, the industry needs more focus on proper training for the users.

People are part of the system and can be a major source of variation. Suppliers need to consider what types of training to provide with their products in order for users to achieve expected performance.

A TEAM APPROACH

Realizing the full benefits of single-use technologies requires an unprecedented level of communication and information exchange among the key players. More collaboration is needed by the drug manufacturers, integrators, component suppliers, and regulators than exists with traditional manufacturing systems. This increased collaboration must work through all aspects of the design, testing, manufacture, and validation of the single-use systems and the drug substances with which they are used for many years after approval. This creates a pathway for industry to share information and to partner at multiple levels. Shared validation for single-use systems is only one step — but a very important step — in developing a greater understanding of the needs and constraints facing the industry and ensuring safe and effective drug products are supplied to patients in need.

Single-Use Systems
for Storing and Shipping Frozen Drug Materials – Every Component Counts

By
Derek Pendlebury
OEM Channel
Manager Bioprocessing
CPC - Colder Products Company

PROCESS FLEXIBILITY WITH EXTENDED REACH AND COST SAVINGS

Freezing in single-use assemblies allows pharmaceutical manufacturers to expand their reach, increase their process flexibility and efficiency, reduce their capital requirement, lower their operating costs by enabling batch processing, and ultimately serve more patients in a shorter time. Presterilized single-use systems require no cleaning or sterilization. Maintenance and validation times are reduced. Large volumes of an expensive biological drug substance can be frozen in batches to allow the drug product to be manufactured based on real-time commercial or clinical demands.

It has become common to geographically decouple global drug substance bioprocessing from final drug product manufacturing: Large amounts of the drug substance can be produced at one site, and then the material is frozen into many smaller units and shipped to different sites for final drug processing. Use of integrated, single-use freeze-thaw systems composed of plastic bio-containers, bags, tubing, and connectors is now standard for the industry.

Although the biological drug material used in cell and gene therapies is technically not a drug substance, it requires the same seamless transportation between sites. Freezing the biological material in single-use assemblies has been the enabling technology for managing the logistics. Autologous cell therapies use the patient's own cells collected in a hospital or clinic, then sent to a lab to be manipulated, concentrated, and then returned to the clinic to be injected into the patient as therapy. Because the patient's cells are the active pharmaceutical ingredient, there is less margin for error for container failures and logistic errors. Time is of the essence, and there may be very little material reserved as a backup. All the components of the single-use freeze-thaw system are part of the solution to improve the health of a sick patient.

DRUG SUBSTANCE INTEGRITY VIA FREEZING AND SINGLE-USE SYSTEMS

Regulations mandate biopharmaceutical product quality be controlled throughout manufacturing, storage, transportation, and delivery to patients. Operations often include freezing and thawing of a bulk drug substance, dilution of that purified substance to a target concentration, filtration, filling into a selected container-closure system, additional processing, inspection, packaging, storage, transport, and delivery. Biologics (large molecule drugs) are particularly susceptible to degradation. Freezing is commonly used to overcome the dilemma. It allows biological integrity of a drug substance to be maintained while an array of logistics can be implemented. And, it can safeguard product quality while waiting for precious downstream processes.

Freezing is better than liquid storage for long-term storage and ship-

ping of the drug substance. The freeze-thaw application can also be expanded to process intermediates as a means of extending hold times between steps. It is easier to maintain temperature-control requirements in a frozen state, and there are fewer interactions between the bulk drug and the container. In addition to reducing product degradation, it mitigates risks associated with mechanical stresses that come from relocating containers from room to room of a manufacturing facility, or country to country within an international manufacturing network.

As biopharmaceutical companies move into new markets and launch new clinical research programs, including drugs involved in gene, cell, and tissue therapies, the cold chain resembles that of bioprocessing, and its integrity is more important and challenging than ever.

Using presterilized, single-use freeze-thaw systems instead of traditional freeze-thaw methods that utilize stainless steel tanks and bottles helps manage the quality of the drug substance. Single-use assemblies reduce the risk of cross-contamination and the complexity of dispensing and manual interventions during freezing, thawing, handling, and shipping. Single-use assemblies can be designed with a shorter freeze-path length, the distance from the edge of a container to its center. This allows more uniform heat transfer between different areas within the total volume of drug material and leads to a more homogeneous mix of biological components and a more stable product. Single-use bags can be conveniently stored in freezers of different styles and dimensions.

EFFECTIVE IMPLEMENTATION

Successfully implementing the freeze-thaw process requires careful testing of the physical and thermal properties of the single-use system, as well as the integrity and quality of the drug substance. Frozen drug substances are usually stored at temperatures ranging from -20° C to -80° C for transportation or in-process holds, and more and more drug manufacturers are moving to the lower part of the range for improved results.

The mechanical properties of single-use assemblies are complex. Attention needs to be paid to the materials used to construct the individual components — the bag, tubing, and connectors, as well as the design and configuration of the system, together with any shell or frame used for support. The assembly needs to be tested under expected and exaggerated conditions used for freezing and thawing to simulate normal and possible unintended conditions such as mechanical or vibrational stress or temperature excursions. If multiple freeze-thaw cycles are anticipated, testing of that parameter must be included.

Peace of mind can be achieved by selecting proven and robust single-use solutions from trusted suppliers. Although the biopharmaceutical company or its contract research organization must do a complete validation of the drug substance in the single-use system used for freezing, they can streamline the process by first understanding their supplier's test parameters and results for the components and single-use assemblies they use. Best results are achieved through a collaborative effort between the scientists and engineers from the supplier and end user organizations: material scientists, process and manufacturing engineers, specialists in film extrusion and plastic molding, quality and validation personnel, and product and system design engineers.

Figure 1. Sterile Plastic Containment Bag for Freeze-Thaw Processing.

Figure 2. AseptiQuik G Connectors.

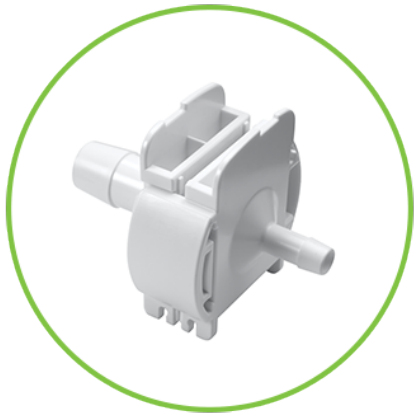


Figure 3. AseptiQuik Connector Body with Protective Barrier.

It takes a well-designed system to ensure a frozen drug substance retains its integrity. Problems that arise from poor implementation come with a significant cost. With single-use systems, plastic material tends to become brittle at low temperatures and must be carefully selected and protected from mechanical stresses caused by handling and shipping. In addition to the single-use container holding the drug substance, the design of the protective shell, tubing and connectors, and secondary packaging must be considered to preserve the precious drug material throughout its journey and life cycle.

CPC ADVANTAGE

CPC focuses its expertise on innovative fluid connection technology important to the single-use assemblies used in the freeze-thaw process. To ensure the assembly functions without failure during storage, transportation, and manipulations before and after, CPC has perfected its seal designs and offers connectors with tested robustness that are also easy to use. CPC's connectors are tested for their intended use, as well as unattended abuse. They have followed the motto "simple is better" in the design of their connectors to explicitly reduce the risk of operator error.

CPC's aseptic connectors marry well with the sterile plastic containment bags (Figure 1) needed for freeze-thaw processing. The connectors allow the end user to make a sterile or aseptic connection in uncontrolled and controlled environments (Figure 2). Each connector half has a protective barrier, usually a membrane, which prevents bacteria and contaminants from entering the fluid pathway while the barrier is in place and opens a sterile fluid pathway once the two components of the connector have been brought together (Figure 3). Bacterial ingress testing is used to demonstrate the ability of the aseptic connectors to make and maintain a sterile connection during use under extreme conditions.

Freezing single-use assemblies puts added stress on connectors. CPC tests its connectors against mechanical stress under freezing and thawing operations within the temperature range of -20° C to -80° C now requested by biopharmaceutical end users. The seal design is tested to make sure it functions as it should and withstands mechanical side-loading, flexing, and tensile forces without compromising the integrity of the seal after the freeze thaw process.

Testing and validation of all raw materials that are used for CPC's connectors is ensured. Extractable testing of the connectors is complete, reliable, and relevant to the downstream needs of the end users. CPC offers genderless connectors, in which the two components that are brought together are identical, thereby eliminating inventory planning and design issues associated with gendered connectors and simplifying the design of a single-use system.

Overall, the adoption of a well-tested, robust, simple-to-operate, single-use connection technology drives a standardized approach to future components and platform designs. Reduced system complexity and production costs are important to biopharmaceutical companies. Decoupling drug substance manufacturing from final drug product formulation and logistically moving biological material for cell and gene therapies are two new paradigms that are only conceivable because of single-use tools that have recently been developed. Single-use assemblies with reliable component parts, such as the connectors supplied by CPC, are paving the way to new possibilities.

When a drop is priceless, **the connector you choose matters.**

When it comes to biopharmaceuticals — you can't afford to fail, so neither can our AseptiQuik® Connectors. They provide quick and easy sterile connections, even in non-sterile environments, allowing you to transfer media with less risk of error.

CPC®

cpcworldwide.com/bio

Extractables Testing
On Single-Use Connectors

By
Ele Vesel

Senior Quality Engineer
for Bioprocessing
CPC - Colder Products Company

Over the last decade, the growing use of single-use technology (SUT) in the biopharmaceutical industry has transformed how drugs are developed and manufactured. Traditional methods using large stainless-steel bioreactors with costly clean-in-place and sterilize-in-place systems have been replaced, in most cases, by more efficient SUT bioreactors. Not only do SUT bioreactors reduce the costs associated with drug manufacturing, but they also offer more flexibility, allowing companies to streamline operations and increase productivity. However, as many benefits as there are to SUT, there is one critical issue drug companies must address when transitioning to plastic equipment, and that is the presence of extractables and leachables (E&L). E&L are defined by the Biophorum Operations Group (BPOG), an industry organization, as follows[1]:

- **Extractables** - A chemical entity that is extracted from a component of a process system into a solvent under controlled conditions that are usually more aggressive than normal operating conditions.
- **Leachables** - A leachable is a chemical entity that comes from single-use systems during normal use.

Testing for E&L and mitigating risks to a product — and more importantly, the patient who relies on it — are essential to being a trusted and reliable supplier in the single-use industry.

THE RISKS OF E&L

The presence of E&L during drug processing can contaminate the final drug product, resulting in reduced efficacy or even threats to a patient's safety. This has led to increased regulatory scrutiny about testing for the presence of these materials. Biomanufacturers submitting a biological license application must include E&L data to demonstrate overall product quality. Yet, specific testing requirements have not been provided by the FDA, leaving the industry with the responsibility to determine the most effective testing methods for ensuring an appropriate evaluation of materials.

A white paper written by members of BPOG titled *Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing* has become an industry guideline for extractables testing by single-use suppliers.[2] While the FDA does not formally recognize the protocol as a regulatory requirement, 20 of BPOG's member companies have adopted it as best practice. The responsibility of testing for the presence of leachables often falls to the end user once product is available to test interaction between the drug and the single-use system. Leachable testing is also done to determine stability and safety of the drug product.

Prior to the creation of the BPOG protocol, there was not an appropriate guideline for reference when it came to extractables testing, leading to a lack of consistency from one supplier's data to another's. This made it difficult for customers evaluating equipment to determine the best fit for their product. The risk-based approach from BPOG drives harmonization and standardization across the industry. As the

protocol states, "Integration of these proposals by SUS suppliers into their existing product lifecycle management processes would be highly beneficial to suppliers to ensure that a comprehensive and consistent set of extractables testing data [is] readily available to biopharmaceutical end users."

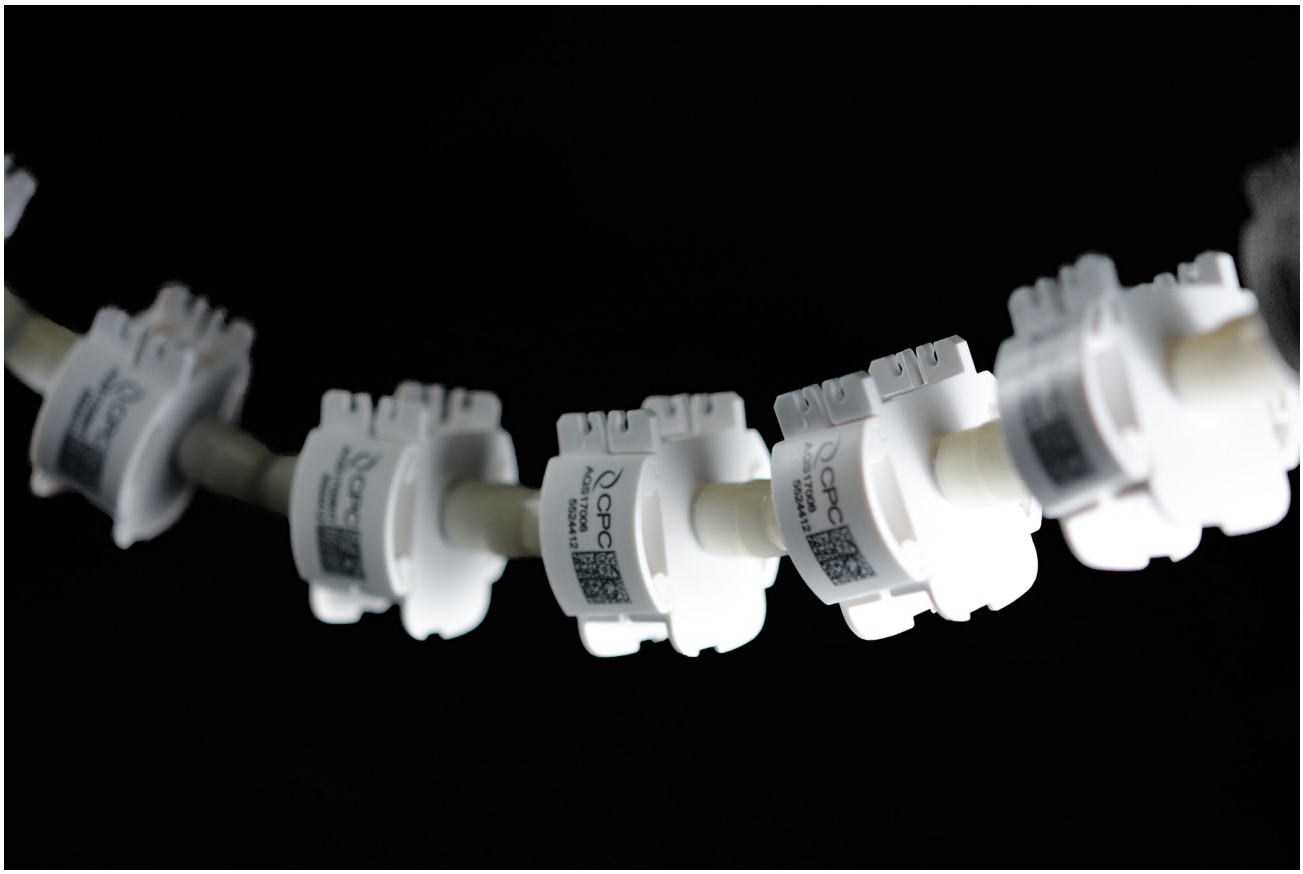
Partnering with a supplier that can demonstrate it has properly used BPOG testing and can provide the necessary data will also help avoid costly delays during the drug approval process. At CPC, it is important we execute the BPOG protocol properly on our single-use connectors, as they are a key component in maintaining sterility throughout processing of our customers' biopharma materials.

TESTING CPC CONNECTORS

To implement this approach in CPC's testing, a resource from BPOG was consulted to select a test lab and complete the evaluation. Testing the connectors became challenging, considering BPOG requirements for surface area to volume. Specifically, the guideline states the fluid used in testing a connector must have a 6:1 surface area to volume ratio. In other words, for every milliliter of fluid used, there must be six times as much square centimeters of surface area. With connectors being such small pieces of equipment, CPC had only one connector that met this requirement, which was its 3/8-inch HB AseptiQuik S. Nevertheless, since all of CPC's connectors are made from the same materials of construction, the results of the testing and accompanying data could simply be adjusted based on the different surface areas of other connectors.

Next, CPC had to determine how to create enough exposure to the connectors in the flow path during testing. Our team decided to create a daisy chain constructed of 20 connector halves (10 connected sets). Each connector had a small piece of PFA tubing attached to the hose between each connector, creating a long series of connectors (in Picture 1 below). The connectors also had to be presterilized prior to connection.

BPOG's protocol recommends testing the connectors with six common extraction model solvents: water for injection (WFI), 0.1 M Phosphoric Acid (low pH), 0.5 N NaOH (high pH), 50 percent Ethanol, 5 M NaCl (high ionic strength), and 1 percent Polysorbate-80 (represents typical surfactant-containing aqueous solutions). The solvents had to be carefully poured in at an angle with breaks during filling to avoid trapping air in the tubing.



AseptiQuik S 3/8" hose barb connector used in testing, representing all AseptiQuik products made of the same materials.

Each solvent was then tested at three different time points (less than 30 minutes at 25° Celsius, 24 hours at 40° Celsius, and seven days at 40° Celsius). A population of connectors was exposed to gamma radiation, and a separate population was exposed to autoclaving. A total of 36 setups were used with 1,440 connectors used per setup. After exposure was complete, the test lab removed the solvents to examine the results and compiled a 120-page report. CPC then created a summary report to share with customers.

Visit the CPC website to request a copy of the report.

INITIAL DATA FINDINGS

After completing testing on its connectors, CPC discovered some key learnings, which included:

- **The 4 other solvents did not find any other extractables that Ethanol and NaOH didn't identify.**
- **Ethanol had more readings at 24 hours than 7 days, which was not the case in the other solvents.**
- **When there was a unique result, it was typically a low concentration (<0.1 µg/cm2).**
- **The less than 30 min time point did not yield any valuable data.**
- **Many readings were very low (near the reporting limit).**
- **NaCl barely had any extractables.**
- **It was very hard to fit all of the daisy chain assemblies into the ovens at the same time.**

While the study setup was quite extensive and may be more than what is needed for a typical connector, it was a valuable exercise in that it did not show high levels of extractables. In fact, the levels of extractables that came from the connector were quite low, especially considering the testing is meant to be above and beyond the normal application conditions.

This data is intended to facilitate any future risk assessments performed by drug manufacturers when implementing a sterile connector. It also provides a better understanding of what these test conditions offer should there be any opportunities for improvements in future testing.

CONCLUSION

As the biopharma industry moves away from blockbuster drugs and toward a more targeted approach to drug development for smaller patient populations, SUT serves as a key tool in modern drug development. While the risk of E&L may present a potential obstacle in the adoption of certain single use components, working with a supplier that demonstrates its commitment to risk mitigation and compliance through appropriate BPOG testing is crucial to delivering safe and effective drugs

Though connectors can be considered a low risk item from an extractables point of view, due to their small surface area and short fluid contact time, CPC understands the value in creating comprehensive data for the industry. This data will prove valuable as the AseptiQuik connector continues to become the standard single-use connection in the industry.

REFERENCES

1. Biophorum Operations Group, Extractables and Leachables, https://www.biophorum.com/resource/extractables-leachables/#0

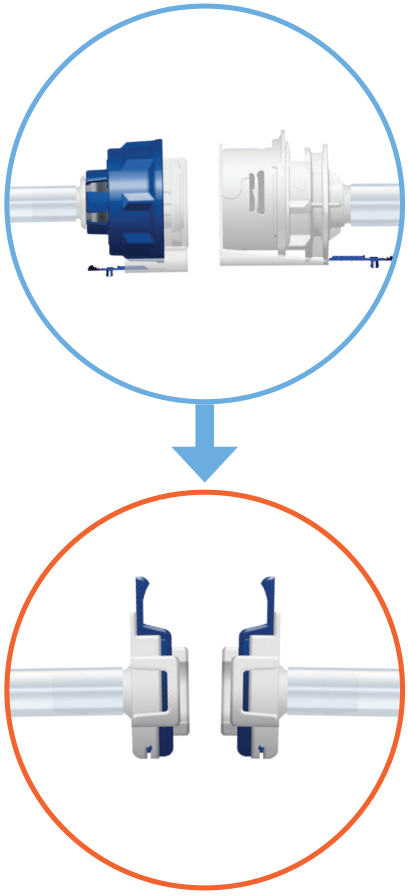
2. Ding, Weibing, et. al. (2014). Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing. Pharmaceutical Engineering. Vol. 34;6 https://www.biophorum.com/wp-content/uploads/2016/10/17_file.pdf

The Path To Genderless Connectors:

How Genderless Sterile Connectors Lead To More Flexibility, Faster Changeovers And Reduced Costs

By
Todd Andrews

Global Sales And Business Development Manager – Bio
CPC



Genderless sterile connectors — with their ability to interconnect with each other without male/female limitations — can enhance the flexibility of single-use systems in a wide range of bioprocessing applications. Genderless connectors reduce system complexity, which in turn lowers requirements for inventory management, simplifies operator training, and reduces misconnections in the manufacturing suite. In fact, it's possible this new connector design will drive industry change similar to the transition from stainless, reusable systems to single-use. How can something as small as a tubing connector have this impact? The answer starts with a bit of history.

Bioprocessing began with reusable stainless steel systems, purpose-built processing plant schemes with steel vessels and permanent connecting piping. This plant approach offered large-scale biopharmaceutical manufacturers the ability to process larger quantities of product, but the downside was twofold: the cost and time associated with building these processing plants and the difficulty of making process changes once the plants were constructed.

As the industry grew, so did the pressures on biopharmaceutical manufacturers to develop more and different drugs, to bring them to market faster, and to reduce costs. Something had to change to facilitate shorter production runs with more changeovers. Single-use systems, consisting of bags, tubing, connectors, and filters, delivered the operational flexibility needed to meet industry demands. One of the benefits of single-use connectors is that design manufacturers can purchase them clean and presterilized, effectively "outsourcing" the cleaning and sterilization activities required in a traditional stainless steel operation. This not only reduces validation and operations expenses, but it also improves the speed and safety of drug development and delivery.

Single-use initially gained acceptance in sterile cell culture media and process buffer storage applications, where the first sterile media bags were used. Following this, engineers developed single-use bioreactors that have quickly moved from research and development labs into pilot plants and production facilities. To illustrate, a recent online survey reported that 50 percent of respondents agreed stainless steel pilot-scale 50L to 500L bioreactors/fermenters are increasingly obsolete due to the emergence of single-use pilot-scale solutions (*Aspen Alert*, April 23, 2015).

For many research and small- to medium-scale operations, single-use systems are the way to go. These facilities need the ability to easily add new

products to the mix, rapidly convert processes, and quickly make process adjustments as needed. Large operations are also seeing the benefits of incorporating single-use systems, and hybrid systems (a mixture of stainless steel components with single-use components) are popular alternatives for manufacturers with existing stainless steel equipment. Regardless of size, all bioprocessors need to be able to adapt processes and execute changeovers while meeting required time-to-market and efficiency goals. There are also a number of drug and contract manufacturers focusing on internal projects to standardize single-use designs across their processes and facilities to reduce the complexity of single-use systems manufacturing.

This brings us to tubing connectors, an often overlooked but critical component in single-use and hybrid bioprocessing systems. Single-use systems need secure, reliable, leak-free connections between various components and processes. These connections are used in conjunction with the silicone or thermoplastic tubing that serves as inlets and outlets throughout a processing system. Single-use connectors can be the first and last line of defense in a single-use system. Even with the best bag, the best filter and the best tubing, it is all pointless without a reliable and robust connector.

Figure 1. MPC/MPX back-to-back adapters give end users the flexibility of connecting single-use systems that feature identical coupling connections at the end of their tubing.

HOW GENDERED CONNECTORS LED TO THE ADVENT OF GENDERLESS

While gendered connectors have been a key building block in the implementation of single-use technology, using typical gendered connectors can lead to unintended consequences that genderless connectors eliminate. Here are some of the real-world problems behind the development of genderless connectors:

Mating Issues

Many processors can relate to the frustration of getting different single-use systems from multiple suppliers with the same gender connections (one system comes in with a male half, and the other system also has a male half). The inability to connect system halves is particularly frustrating because the problem often goes unnoticed until the point of use. This means the user has to quickly create some sort of adapter piece to go between the two systems because the connection is needed *now*.

For instance, if a processor received the two systems as described above (each system with a male connector half), he or she would need to obtain two female connectors, a short piece of tubing, and something to secure the tubing to the connector (i.e., cable tie, barb retainer, etc.). Then the processor would need to assemble the two female halves to the tubing, secure the tubing, and autoclave the final assembly in-house. This time-of-use jerry-rigging leads to several negative impacts for the processor:

- Adds material and labor costs to create the adapter assembly
- Adds labor costs to assemble the adapter
- Adds costs for autoclaving the assembly in-house
- Delays the usage of the single-use systems until the adapter has been created and sterilized
- Introduces additional risk of leaks or contamination

Another way to deal with the same-gender issue is to use a connector adapter, such as the back-to-back adapters available from CPC (see Figure 1). However, this solution requires anticipating the problem — and adapters are not presently available for gendered sterile connectors.

Inventory Issues

Any time a component requires a specific mating component, finished-goods inventory needs can more than double, or even triple. As an example, consider an assembly as simple as a basic transfer line with sterile connectors on each end. There are three possible configurations depending on the application setup: a female-to-male version, a male-to-male version, and a female-to-female version (see Figure 2).

Other inventory issues include:

- Increased ordering complexity because the processor needs to define which tube set works with which part of the application
- Greater demands on single-use systems manufacturers because they need to be able to produce and stock three different assemblies
- Longer lead times because systems suppliers are making lower volumes of multiple SKUs as opposed to higher volumes of one SKU
- Increased stocking requirements for end users

In contrast, using genderless connectors on the same tube set results in only one possible tube set.

MOVING FROM GENDERED TO GENDERLESS STERILE CONNECTORS

The inherent simplicity of genderless sterile connectors is easy to grasp, but the idea of transitioning to genderless raises questions from systems designers and processors. Here are some frequently asked questions:

- ***What obstacles might I encounter when transitioning to genderless connectors?*** One obstacle can be the design of the genderless connector itself. It is critical to select a connector that is truly easy to use,

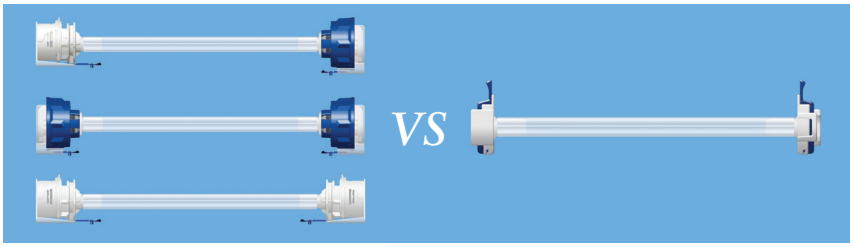


Figure 2. Female-male, male-male, and female-female tube sets generate three times the SKUs as genderless tube sets, adding inventory costs and increasing complexity. In contrast, genderless connectors enable stocking just one tube set.

robust, and does not require additional hardware to assemble. Some connectors are not easy to use because they require many assembly steps to make the final connection; the more steps there are for assembly, the higher the chance an operator will make an error. If the connector requires hardware not integral to the connector to secure the connection (e.g., tri-clover clamps), operators can misplace or forget to install the added components. A reliable connector should not need additional hardware, and an easy-to-use, robust connector has the greatest chance of being used correctly.

- ***What needs to change in my facility to make genderless connectors work?*** If a processor is already using another connector, the biggest obstacle is the change control process. Even though something can be significantly better and provide efficiency savings, the process of revalidating a new connector can be cumbersome and slow. Once validated, however, the change to genderless connectors usually results in process improvements, cost savings, reduced training requirements, and fewer quality issues on the manufacturing floor.
- ***Can I mate two genderless halves that have different hose barb sizes?*** Yes, as long as the connectors are from the same product family. CPC's genderless design allows this type of connection. For instance, one side with a ¾" hose barb can mate with a ¼" hose barb on the other side. This also includes mating a ¾" sanitary to a ½" with a hose barb. This capability eliminates the need to install reducer fittings somewhere else in the system, an extra step that increases component and labor costs.
- ***Are genderless connectors a step toward standardization?*** It is next to impossible to attend a conference on single use without hearing about standardization. While standardization can cover several topics, one of the most common discussion topics is connector compatibility (e.g., interchangeability). Research reported in BioPlan Associates, Inc., April 2014, Biotechnology Industry Council™ Analysis of Single Use Connectivity showed that 88 percent of respondents viewed standardizing connector compatibility as important. This same study reported a 73 percent preference for genderless connectors over gendered connectors. End users are identifying easy-to-use, robust genderless connectors as an answer for both standardizing single-use systems and eliminating many of the headaches experienced at facilities using single-use technology.

CPC's interchangeable AseptiQuik® genderless connectors enable tubing transitions between tubing of different sizes, from ¼" to ¾" flow.

APPLICATIONS FOR GENDERLESS CONNECTORS

Genderless connectors can be utilized in all transfer applications where gendered sterile connectors are found, including suite-to-suite, seed train, and formulation/final fill. The difference is that genderless connections significantly reduce system complexity in all of these processing applications. Because component configurations within transfer lines vary as much as the options for tubing, connectors, and filters, viable connection technologies require flexibility. Multiple termination options provide the flexibility needed to meet today's mounting and flow requirements. For instance, a basic transfer line could be as simple as silicon tubing with two genderless sterile connectors, or it could be more sophisticated, incorporating tubing, SIP connectors, sterile connectors, and sterile filters.

When changeovers need to occur, end users can pull stock and connect components and processes with genderless connectors, resulting in increased flexibility. No matter the processing stage, genderless connectors provide maximum flexibility.

CONCLUSION

Just as single-use technology emerged in response to marketdemands, so too have genderless single-use connectors. Systems designers and processors have maximized the benefits of single-use and hybrid processes — those of increased flexibility, faster changeovers, and reduced costs. Now the bioprocessing industry can also benefit from the reduced components complexity afforded by genderless connectors.

About CPC

CPC (Colder Products Company), the leader in single-use connection technology, offers a wide variety of bioprocessing connection solutions. Our innovative designs offer flexibility to easily combine multiple components and systems including process containers, tubing manifolds, transfer lines, bioreactors, and other bioprocess equipment. AseptiQuik® Connectors provide quick and easy sterile connections even in non-sterile environments — a critical capability for biopharmaceutical and bioprocessing manufacturers. Featuring a wide range of options including 1/8- to 1-1/2-inch sizes and genderless and gendered connections, AseptiQuik connection technology delivers sterile, high-quality single-use connections and easy media transfer with less error risk. For additional information, visit cpcworldwide.com or call +1-800-519-7633.