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IMPROVING THE INTEGRITY OF PHARMACEUTICAL STERILITY TESTING:

A NEW ROBOTIC APPROACH

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ABSTRACT

Concern over the occurence of false positives within the sterility testing process has been and will continue to be of grave concern to pharmaceutical manufacturers. To date, very few truly effective solutions to this problem have been offered, while the FDA becomes ever more stringent over false positive results and second phase testing.

A new robotic system for sterility test applications is described, which completely isolates the actual test area from human operators, and thus reduces substantially any possibility for false positives. The configuration utilizes the USP-preferred membrane filtration method, and uses the Millipore Steritest filtration chambers as its "output" for incubation. The system allows a technician to simply place product to be tested in an input station, mount the Steritest cannisters in designated sockets, then remove the empty containers and filled cannisters post-processing and incubate. This new method allows for the testing of a variety of different sample containers, including a range of vial sizes, ampoules, and pre-filled syringes.

The test methodology will be described in detail, including an in-depth look at the actual application procedure and a discussion of major system components and overall system configuration.

INTRODUCTION

Sterility testing was first documented in the British Pharmacopoeia in 1932, and several years later in United States Pharmacopoeia (1936).[1] While sterility test methodology has advanced significantly in the ensuing fifty years, most sterility testing is still performed manually. Even with the employment of elaborate gowning of technicians, laminar flow hoods, and highly sophisticated aseptic techniques, false positives remain a serious concern to pharmaceutical manufacturers.

While it is well understood that the testing of 20 samples from a large batch of product does not necessarily offer conclusive evidence of batch contamination or sterility, it is nontheless an important indicator of potential problems within the aseptic manufacturing process. Unfortunately, it is often the case that false positive results from sterility testing are significantly more frequent than *true* positive results. As such, an organization is forced to spend an inordinate amount of time and resources investigating the initial sterility test failure and performing the required retest. All the while, of course, the product must be quarrantined, taking up valuable warehouse space and delaying - perhaps substantially - the scheduled shipment of product.

These circumstances will inevitably lessen the productivity of both lab and manufacturing personnel (forced to spend valuable time on an investigation), and may result in cancelled orders due to late (quarrantined) shipments, or possibly even the loss of product due to eventual rejection. While the latter is relatively rare, it is evident that the occurrence of a false positive result may still be quite costly; a false positive investigation may be easily translated into actual dollars lost due to reallocated internal resources.

THE PROBLEM

In a survey published in the November/December issue of the Journal of Parenteral Science & Technology, over 57% of respondents reported that their first stage sterility test contamination rate was above .25%, while over 27% reported a first test contamination rate of over 1.0%.[2] These numbers may not seem excessive, especially when compared to contamination rates of a decade ago. However, there are several points which should be emphasized here:

1. Even when a false positive rate is brought down into the .25% range, an organization performing an average of 2000 tests per year is still experiencing approximately 5 false positives annually. When the percentage is 1.0%, the number of

false positives/year jumps to 20. Though all too rarely calculated, the actual cost of false positive investigations, retests, and shipment delays are substantial.

2. If just one false positive cannot be proven false during the retest, the batch must be rejected. The cost of such an occurrence can be devastating: batch rejection results in the total loss of revenues equal to the product's market value.

3. The survey reported the responses from only 33 well-known and highly respected parenteral drug manufacturers. It could probably be safely assumed that there are many other manufacturers, not included in the study, whose false positive rates are significantly higher. As such, the average dollar risks are probably considerably higher as well.

4. A final factor to be considered is the FDA's increasing stringency over retest rates, policies, and procedures. Over the past decade, the agency's position on what might be seen as an "acceptable" false positive rate has become ever more conservative; what was satisfactory several years ago is now completely unacceptable; what is viewed as tolerable today may well be labeled excessive tomorrow.

The actual problem, however, is not false positive rates, or the ensuing resource commitment required to rectify a false positive. Rather, the problem is how to avoid false positives altogether. Even when the best possible aseptic technique is employed, false positives *may still arise*. The reason is simple: exposure of the test materials and process to human technicians, resulting in adventitious microbial contamination.

The solution is equally simple: the complete removal of humans from the application.

NEW APPROACHES TO STERILITY TESTING

Over the past decade or so, there have been a number of significant advances in sterility test technology. The first, and perhaps most significant, is the development of the closed system. Using this technology, manufacturers have dropped their sterility test failure rate substantially. The physical advantages of a closed system are obvious: the greatly reduced possibility for generated human contamination to enter the test process. In other words, while the closed system does not reduce the presence of technician-generated contamination in and around the test area, it does block such "bugs" from entering the membrane filtration chamber.

This is indeed a giant step forward. However, the closed system method does have an achilles heel: the unexposed needle used to extract product from a given container. While this may seem trivial to an observer, those actually involved in the procedure are well aware that it is not. This seemingly minute opportunity - brief in duration though it may be - is all it takes for a single microorganism to contaminate the test process.

Another new technology is the isolation chamber; a large, "bubble" or "body glove box" which allows space-suited technicians to perform the application in a self-contained plastic bag. While this has proven to be fairly effective, many have called the technology only a "temporary" solution due to a couple of factors: primarily cost and complexity.

First off, the base price of these systems is quite high due the required integrity of the plastic chamber, various required accessories, and the inherent cost of the complex seal mechanisms, or transfer ports. Second, the complexity (and cost) of the product's scheduled maintenance procedures is often inhibitive, while the use of pericedic acid and other hazardous substances make the re-sterilization procedure a potential health risk and even impossible in some areas where the disposal of such substances is highly regulated. Third, as a result of the acid treatments, the product lasts a very short time; after five years, the material begins to degrade. This, of course, makes the technology *very* expensive.

What is needed, then, is a system which can provide the advantages of both a closed system and an isolated chamber - without any of the above-listed disadvantages.

ONE SOLUTION

Several years ago, Hoffman LaRoche developed a system which met these criteria: a robotic system which automates sterility test applications using a closed system within an isolated area. This configuration, using the Millipore Steritest™ system, was designed such that all contaminant-sensitive operations would be performed away from human exposure.

On a conceptual level, the robotic system described within this paper shares many characteristics with the LaRoche configuration, with several important exceptions:

1. Flexibility: the initial LaRoche system was designed for the sterility testing of SVPs, or smaller vials. Eventually a second system was developed for ampoules. Currently, a third system is coming on line for pre-filled syringes. The robotic system described below is capable of automating all three container types simultaneously. That is, one configuration allows for the consecutive testing of any one of the three containers - without modification, reconfiguration or reprogramming.

2. Availability: The system described herein is a complete, turnkey system commercially available as an "off-the-shelf" product. The LaRoche system, on the other hand, is a custom system initially designed solely for in-house use, and not for resale.

3. Size: The new system is designed for installation into a variety of customer clean room environments. As such, it offers a very small "footprint" of only 38" x 74", and takes up very little clean room floorspace.

In short, while the Hoffman LaRoche system is an excellent first generation effort, the robotic system described within this paper picks up where LaRoche left off, providing a "next generation" system which offers parenteral drug manufacturers a flexible, practical, and cost-effective solution for sterility test applications.

A NEW ROBOTIC SYSTEM

The following pages describe the design and specifications of a turnkey robotic system for the sterility testing of parenterals (figure #1). This system provides complete, automated execution of the sterility testing process with operator intervention only during system set up and test completion. It is believed that the system will reduce the possibility of obtaining false positive results by a substantial margin over manual methods.

The system is based on a "clean room" robot, designed specifically to inhibit microbial as well as particulate contamination from entering the test process. Laminar Flow patterns in conjunction with special materials and clean room mechanisms have been optimized to prevent the introduction of particulates into the process.

The system utilizes the USP-preferred membrane filtration method, and uses the Millipore Steritest filtration chambers as its "output" for incubation.

Overview of System Operation

The system performs all aspects of the test sequence. An operator simply places product to be tested in an input station, loads the Steritest chambers, tubes and needle in designated mounting fixtures, then removes empty product containers and Steritest cannisters post-processing and incubates. The robot performs all container manipulation, product extraction, rinsing (if required) and media filling. A variety of different sample containers may be used, including a range of vial sizes (SVPs) and ampoules (please see Appendix A & B for detailed notes on system operation). Pre-filled syringes may also be accommodated through the use of an optional syringe mounting module. The Millipore Steritest expendables may be used with the system with no modification.

System Description

The robotic system consists of a five-axis clean room Robot and system controller, two drawer-type input stations (each designed for lots of twenty product containers), dual peristaltic pump units, a container decapping mechanism, an ampoule opening station, and a septum sterilization and quick-dry station (figure #2). The system is controlled via a teach pendant connected to the system controller, with all necessary application programs provided. All components are mounted on a stand-alone system frame. A laminar flow hood, approximately 6' x 3' is mounted over the system, providing HEPA filtered vertical laminar flow at all times.

The Robot

The robot is designed specifically for use within Class 10 clean room environments, and is used extensively within the semiconductor industry for a variety of class 10 semiconductor clean room applications (see Table #1).

The robot utilizes specially sealed body parts and arm joints, preventing the escape of particulates above the work surface. The robot is mounted on a linear track, providing access to the entire surface area of the work station. The track is mounted to the side of the system frame, with all drive mechanisms isolated by a vertical wall from the test area; further reducing the introduction of particulates into the process.

A unique dual linkage arm-support structure allows the robot to achieve smooth linear motion in any axis, thus reducing vibration, increasing arm rigidity, and further reducing particulate generation.

In addition, the entire arm is evacuated via a negative air pressure system which pulls air and any generated particulates through the arm and exhausts outside the clean room area.

The end-effector, or gripper, is designed to adapt to and manipulate a variety of containers and container sizes. It is constructed of annodized aluminum with stainless steel "fingers." All other exterior components are constructed of sterilant- resistant materials, allowing the system to be completely sterilized prior to system operation.

System Controller and Teach Pendant

The system controller controls and monitors all application programs. The unit is powered by an 8088 central processor with twin 8085's supporting motion control. The controller housing contains all necessary firmware and hardware for operating the system and defining new application routines. A floppy drive is provided for the easy storage and retrieval of application programs. Software (application programs) is provided.

The teach pendant, or "CommKey," is a special keyboard cabled to the system controller. The CommKey provides full operator control over system operation.

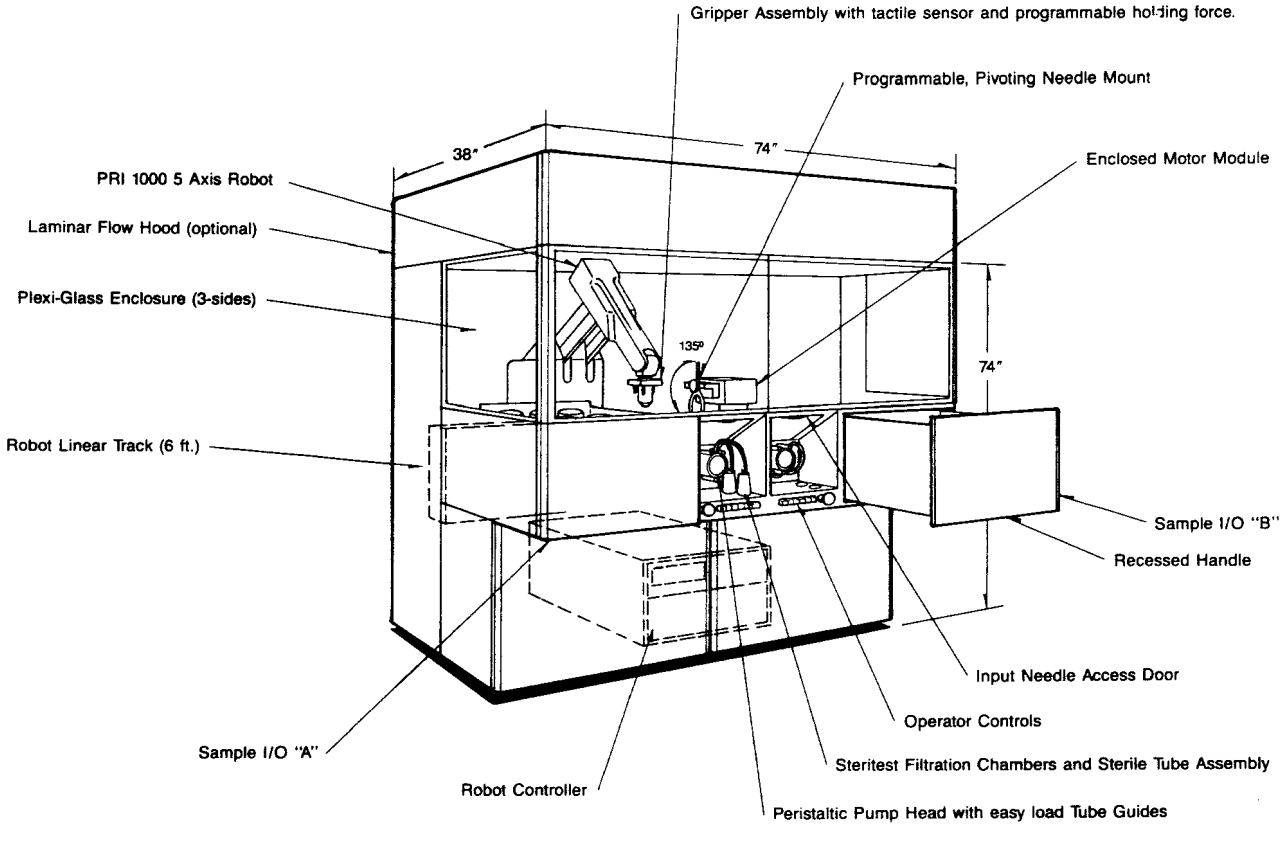

FIG. 1 PRI ROBOTIC STERILITY TEST SYSTEM

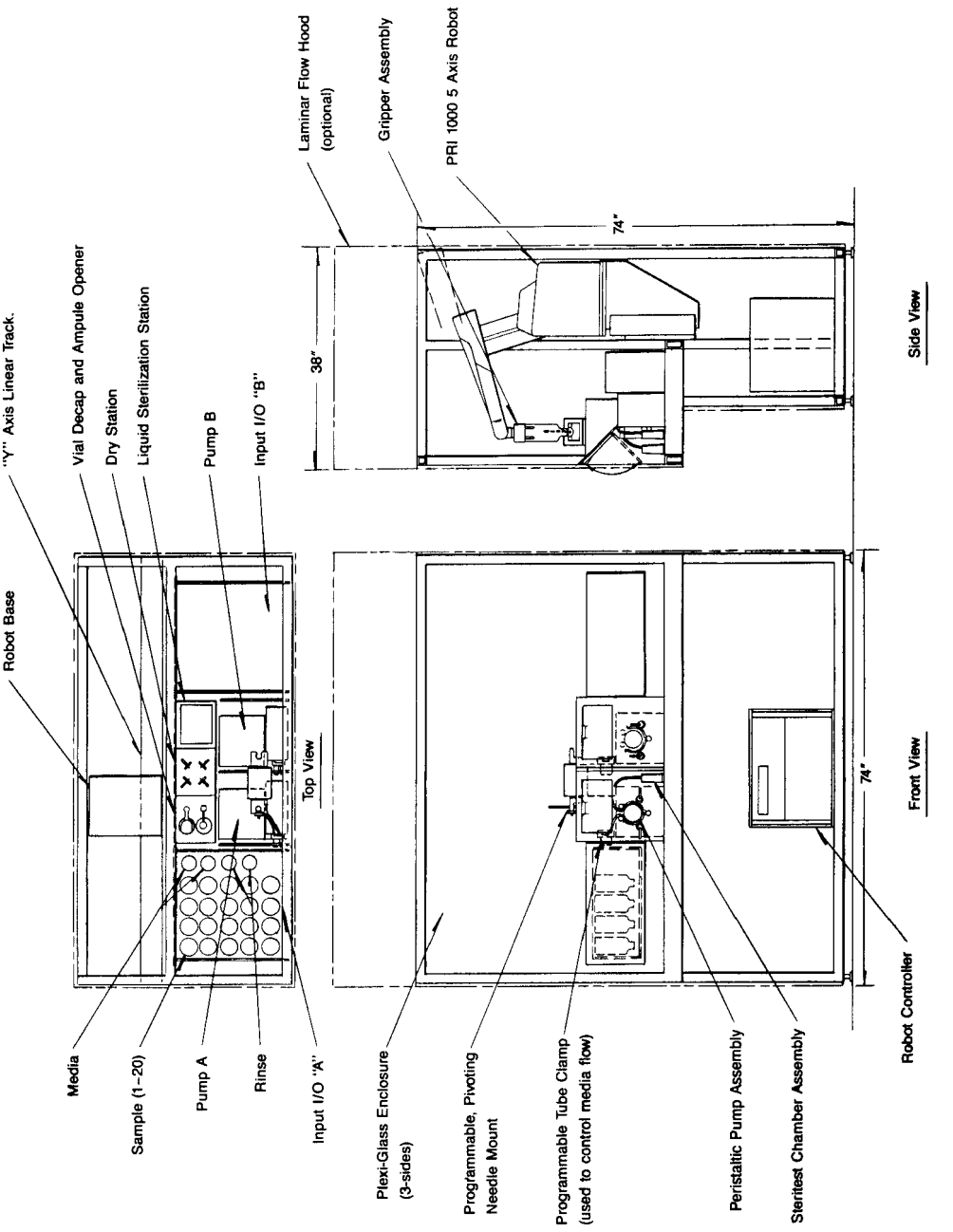

FIG. 2 PRI ROBOTIC STERILITY TEST SYSTEM

Table #1
Clean Room Robot - General Specifications:

Maximum Reach (body to flange centerline):	18.3"
Maximum Payload:	15 lbs
Resolution (smallest incremental step):	0.001"
Repeatability:	0.002"
Maximum Speed (point to point):	20.6"/sec

Table #2
General System Specifications

Container types:	SVPs Ampoules *Prefilled Syringes (optional)*
Input Stations:	2
System Throughput:	Up to 2 lots (40 containers)/Hour*
Dimensions:	74" x 38" (Footprint)
Facilities Required:	
Electrical:	115V, 50/60 Hz 10A (230V, 50 Hz optional)
Exhaust:	Negative Air Pressure (minimum flow)

* *Estimated. Actual application throughput will depend upon the size of the product containers, the viscosity of the product, and the specifications of the peristaltic pump.*

Product Input Stations

Two input stations are provided for the introduction of parenteral products into the test area. The stations are drawer-type mechanisms fitted with fixtures for holding racks of twenty product containers, two containers of incubation media, and three containers of rinse solution (all SVP/LVP containers would be introduced upside-down). The bottom of each drawer is perforated for optimum laminar air flow down around the product containers and out through the bottom of the input station.

Peristaltic Pump Units

Two peristaltic pump units are located between the input stations. The Steritest cannisters are placed in sockets next to each pump. The cannister tubes are threaded through the pump mechanism using a special easy-open/close clamshell design, through a programmable tube clamping device, and up through a hatch above the pump area, with the capped needle mounted on a stainless steel bracket just up inside the test area.

Sterilization/Quick-Dry Stations

These stations, located in the rear of the test area, prepare all containers prior to the actual test sequence. The sterilization station allows for both vial septums and ampoules to be bathed in an appropriate solution (alchohol/iodine). The quick-dry station levels a stream of ultra-clean air at the vial/ampoule to dry the container.

Ampoule Opening Station

An ampoule opening station is also located at the rear of the test area. Pre-scored ampoules would be taken to this station after sterilization and opened; the top would be delivered to waste; and the ampoule would then be delivered to the test area.

Needle Mounting Bracket

A programmable, pivoting, stainless steel needle mounting bracket is located in the test area. This allows for a needle to be positioned vertically for vials (SVPs), and at a downward, 45 degree angle for ampoules. The needle mounting area is designed for optimum laminar air flow, while the mounting procedure is designed such that the uncapped needle may *never* be directly exposed to a human operator.

CONCLUSION

The new robotic sterility test system overcomes several major drawbacks associated with current, commercially available methods. Since the robotic system uses a well-proven closed system methodology in combination with the total isolation of the test area, false positive rates should drop by several orders of magnitude or even be eliminated completely. It is worth noting that the Hoffman LaRoche configuration has not reported a single false positive since its installation - approximately 2 1/2 years ago.

As with existing methods, the new system does require thorough cleaning & sterilization of system components and surfaces. However, during the actual application, the system is substantially less dependent on the technician's aseptic technique. As such, the product not only enhances the integrity of the sterility test process, it also greatly increases user confidence in test results. This, of course, is a benefit which is very difficult to ignore.

Notes:

[1] *Olson, W.P., and Groves, M.J., Aseptic Pharmaceutical Manufacturing, Interpharm Press Inc., Prairie View, IL, 1987*

[2] *Akers, J.E., Carleton, F.J., Clements, W.C., and Woods, J.A., "Survey on Sterility Testing Practices," Journal of Parenteral Science & Technology, volume 41, number 6, 197-206, November/December, 1987*

Appendix A

SYSTEM OPERATION - Vials:

OPERATOR:

1. Operator pulls open input station #1, places the appropriate rack of product, media and rinse solution (if required) into the drawer, then closes the drawer.
2. Operator places Steritest cannisters in appropriate sockets; threads tubes through peristaltic pump unit and programmable tube clamp; opens hatch, mounts needle on bracket, closes hatch.
3. Operator initiates appropriate application program via the CommKey.

ROBOT:

4. Robot removes cover from needle.
5. Robot removes product container from rack, brings to preparation area and decaps.
6. Robot places tip of container into appropriate sterilization bath for specified period. Tip is then air dryed via clean air blast.
7. Robot brings container to test area and down onto needle, puncturing septum.
8. Robot activates peristaltic pump. Substance is extracted.
9. Robot deactivates pump, replaces empty container in original rack position.
10. Robot repeats steps 7 - 9 until all product containers have been extracted.
11. If required, Robot repeats steps 7 - 9 with rinse solution.
12. Robot then adds media to each Steritest chamber, performing all appropriate tube clamping and chamber manipulations. Robot returns final media container to original rack position, and signals operator of test completion.

OPERATOR:

13. Operator then removes and prepares Steritest cannisters for incubation.

NOTE: *(All Container Types): During the robot's execution of steps 4 - 12, operator would repeat steps 1 - 3 at opposite Test Station. Execution of test procedure would then be repeated in a cycling fashion.*

Appendix B

SYSTEM OPERATION - Ampoules:

OPERATOR:

1. Operator pulls open input station #1, places the appropriate rack of product, media and rinse solution (if required) into the drawer, then closes the drawer.
2. Operator places Steritest cannisters in appropriate sockets; threads tubes through peristaltic pump unit and programmable tube clamp; opens hatch, mounts needle on appropriate bracket, closes hatch.
3. Operator initiates appropriate application program via Commkey.

ROBOT:

4. Robot removes cover from needle. Needle is then automatically rotated into position at 45 degree downward angle.
5. Robot places first ampoule into sterilization bath for specified period. Ampoule is then air dryed via clean air.
6. Robot brings ampoule to preparation area and opens, delivering top of ampoule to waste.
7. Robot brings ampoule to test area; moves ampoule such that needle is fully inserted into container.
8. Robot activates peristaltic pump. Substance is extracted.
9. Robot deactivates pump, delivers empty container to waste receptacle, etrieves next ampoule.
10. Robot repeats steps 7 - 9 until all product containers have been extracted.
11. Needle is then automatically rotated back to vertical position. If required, Robot repeats steps 7 - 9 with rinse solution.
12. Robot then adds media to each Steritest chamber, performing all appropriate tube clamping and chamber manipulations. Robot returns final media container to original rack position, and signals operator of test completion.

OPERATOR:

13. Operator then removes and prepares Steritest cannisters for incubation.

NOTE: *(All Container Types): During the robot's execution of steps 4 - 12, operator would repeat steps 1 - 3 at opposite Test Station. Execution of test procedure would then be repeated in a cycling fashion.*