Developing Practical Single-Use Processes for New Vaccine Formulations



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New vaccine process designs – and all the kinks that go with them – are typically hammered out in a small scale capacity, for example, prior to manufacturing for early phase human clinical trials. They are then upsized and further defined for industrial scale to supply the vast market. Single-use technologies (SUTs) have been a hot topic for several years now and their advantages well-known: easy product changeover, processing in lower classification areas, reduced CAPEX, elimination of glass, sterility assurance, to name a few. In vaccine manufacturing, SUTs are used throughout the processing stages, from cell culture all the way to filling (Figure 1). SUTs are quickly and conveniently designed, purchased and implemented for short-term manufacturing of clinical trial phase materials. Here a large percentage of new vaccines in Research and Development do not even make it to market.

As the final production stages are critical as they are the last stages before patient injection, the scope of this article covers SU applications involving drug substance formulations, adjuvant processing, final bulk formulation and filling. The actual process itself may include some or all of the following: filtration, pumping, ingredient addition, mixing, adsorption, filling, labelling, sampling and storage. In this paper, only liquid formulations ("presentations") will be discussed.

In order to verify manufacturing success, process testing and control must be performed at the various process stages. To begin any experimental work to verify the entire process or aspects thereof, proper tests must be available that are capable of measuring critical product and process attributes. Of course, materials and parts selections must be considered carefully to ensure components are compatible with both product (i.e., leachables) and process (i.e., vapour hydrogen peroxide). All of these aspects contribute to the development life cycle.

In some cases, the initial design (including equipment, processing flow and parameters) need to change to accommodate single-use. Two examples will be given below, one looking at modifying process aspects in SU to ensure low particulates generation and the other involving a single-use (SU) process to better filter a difficult ingredient. The way in which single-use technologies are designed, verified and monitored, has become more advanced and, therefore, process optimization and changes may need to occur to fall within certain industry guidelines and expectations. A third case study will be given to demonstrate how testing of single-use technologies at

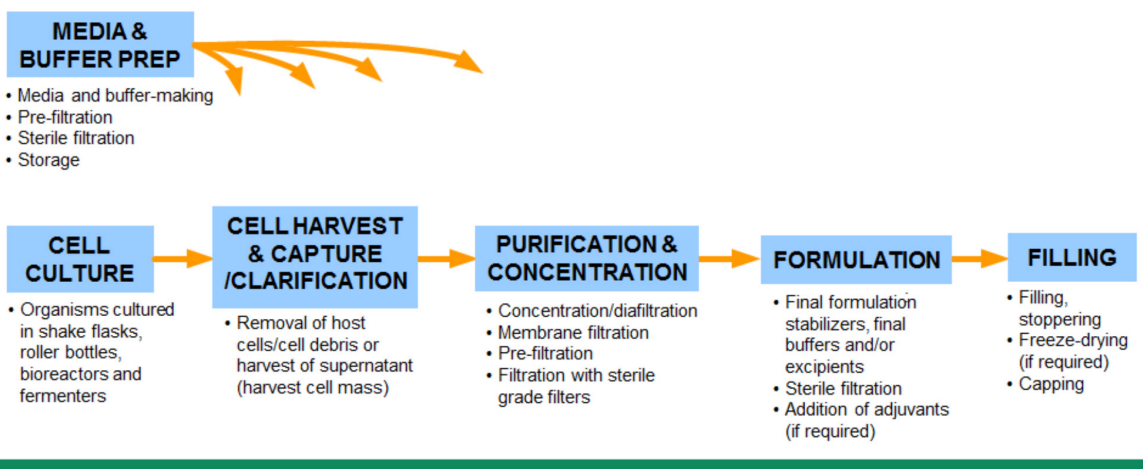

Figure 1: Single-Use Processing Applications in Vaccine Manufacturing

the site, by the end-user, can play an advantageous role in the process monitoring and control.

The following studies highlight experiences and challenges obtained as well as summarize the need, methodology, outcomes and any modifications to the processes to obtain improved outcomes.

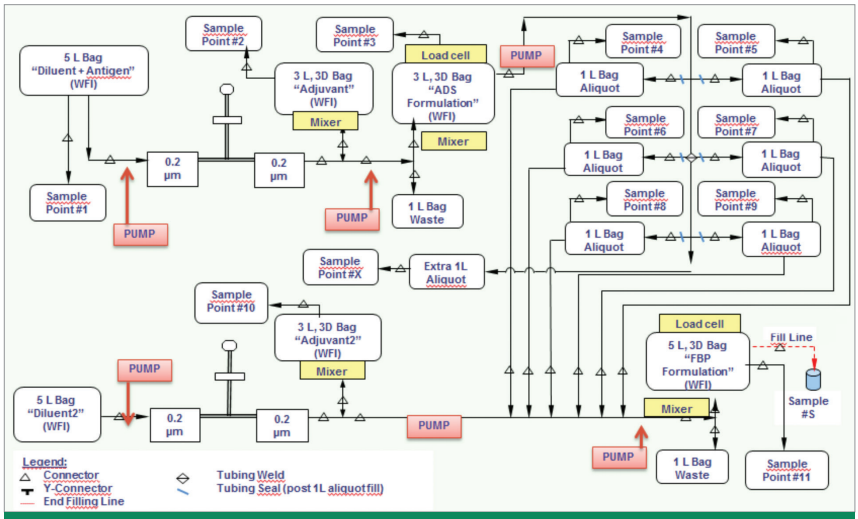

Figure 2: A Worst-Case Process Diagram of Particulates Sampling throughout the Adsorption, Final Bulk and Filling Manufacturing

Parting with Particulates

Prior to the last couple of years, there was minimal guidance on testing, controlling and monitoring particulates, specifically for biopharmaceutical applications in SU. Recently, we now have the BPSA guideline (BPSA, 2014) and the new draft USP <661.3> Plastic Components and Systems used in Pharmaceutical Manufacturing (USP, Draft 2016) which will likely provide much more information on this topic.

Particulates can become complicated in final drug product as they can be either intrinsic or extrinsic. They can originate in the vaccine product (i.e., aggregation), from the product in contact with the process/packaging materials/components, and/or from the vaccine process, environment and storage conditions.

When designing SU systems for vaccine products, generally, suppliers and end-users are expected to control the particulates during the manufacturing and at least meet USP 36, Chapter <788> Particulate Matter in Injections (USP, 2012). Note: there are similar limits for JP 6.07, EP 2.9.19 and WHO IP (5.7) pharmacopeial equivalents, however, for the purposes of this paper, the USP <788> will be referenced. Suppliers typically provide particulates data for the purchased individual SU components or assemblies. As a best cGMP practice, they ensure particulates controlled in the process (i.e., use of cleanrooms, low particulate generating equipment, washing parts, where possible).

Information should be available to support that the material going into the SU assemblies and operated under the end-user's processing and storage conditions does not produce compatibility issues that can impact particulate levels. A combination of supporting information can include, but is not limited to:

- chemical inertness of the SU components after sterilization,
- particulate characterization and stability data of the product after processing and storage in the SU components/assemblies, and
- extractables/leachables of the components and/or assemblies

End-users typically conduct tests to count, minimize and control particulates in the process stages where the SU system is fully assembled, connected and setup with the user's equipment, process, staff, and run under representative or worst-case conditions. The rest of this section summarizes how the end-user followed the USP 36, Chapter <788> Particulate Matter in Injections method (USP, 2012) to perform such a study.

Method

In preparation for cGMP Phase I/II small scale clinical manufacturing, a worst-case process manifold was setup to represent adsorption, final bulk and filling manufacturing of an adjuvanted vaccine at small scale (Figure 2). This closed, SU system manifold was comprised of several assemblies connected together by the end-user. The process was mapped to sample WFI after it had been drawn through the lines at various points in the process such that individual process steps, where particulates may be higher, could be identified and optimized. As well, the purpose was to quantify the accumulation of particles in the single-use components from ingredient addition during formulation to final filled product. Initially, the experiment was run without any optimization of the process to gain a baseline understanding and run it again after optimization.

A light obscuration method was used as per USP 36, Chapter <788> (USP, 2012) for bulk formulations with nominal volumes of greater than 100 mL. Samples at volumes greater than 25 mL were aliquoted into 4 x 5 mL preparations for testing, where the first aliquot was discarded and the last 3 aliquot measurements were

Particle Size (µm)	Allowable Number of Particles per mL
Particle Size < 10 microns	N/Ap
Particle Size ≥ 10 microns	Not more than 25 particles
Particle Size ≥ 25 microns	Not more than 3 particles

Table 1: USP <788> Allowable Limits for Bulk Product Particle Quantities

averaged to obtain a cumulative particle count per milliliter. Relevant particle sizes for examination were 10 and 25 µm such that the limits are shown in (Table 1).

Outcome

After the initial pre-optimized process was run, the counts were higher in three main areas of the process as can be seen in Figure 3. The first was observed in many of the samples where syringes were used to withdraw WFI by piercing them through a sample septum port on the sample bags. Irregular particulate counts occurred for these types of samples. The second showed that the magnetic force generating the stirring mechanism on the SU containers was too strong, generating large particulates. The third was as a result of the peristaltic pump, used to pass product, chewing the inner wall of the plastic TPE SU tubing, leading to spallation and particulate generation.

A new trial was run post-optimization. The WFI representing "product" passing through the assembly shown in Figure 5 was exposed to a similar process flow to the first trial however, the sampled conditions were the ones which would give fewer particulates but still considered more worst-case for mixing times. For example, Figure 3 has XA to XD which are samples with increasing mixing times, while Figure 5 has only XC as this was the tested maximum time the product could be mixed with acceptable data based on the results from the first experiment. The method of sampling was modified so that the samples were withdrawn directly by opening the syringe port/luer lock connection on the sample bags (which was representative of the actual sampling process) (Figure 4). A weaker, yet still effective, magnetic stirring mechanism was used in combination with capped mixing times. A combination of optimized pump head tubing size, tension setting and flow rate (with a longer-term initiative of changing the tubing type at the pump) was applied. With these changes, the study was repeated and all the particulates results passed the USP criteria as can be seen in (Figure 8). It should be noted that a small increasing trend in count for both the 10 and 25 µm sizes was observed during the filling process which can be explained by the fact that the peristaltic pump used to fill the product wears the platinum-cured silicone tubing over time (Saller et al., 2014). This was expected and, considering the process was run under worst-case conditions, the accumulated count of particulates at the end of the process was well within the acceptance criteria.

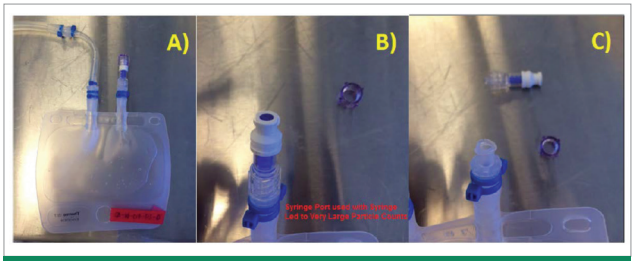

Figure 4: Sample Syringe Port and Orifice. A - Sample Bag, B - Syringe Septum Port, C - Sampling orifice with unscrewed syringe port used after optimization

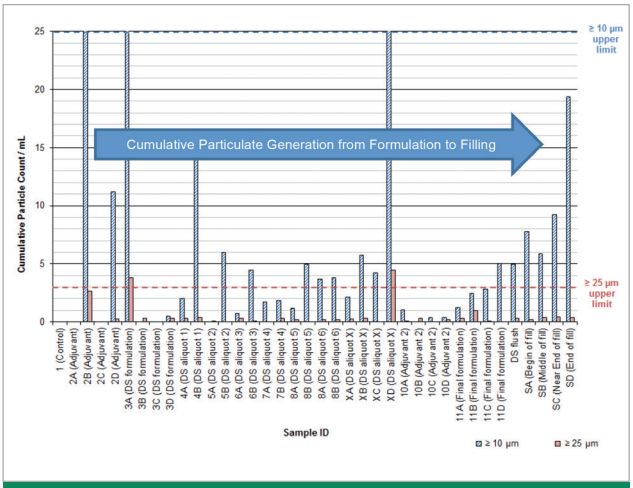

Figure 3: Cumulative Particulate Counts of Formulation and Filling Processes by Light Obscuration as Compared with USP<788> Particle Count Limits – Before Optimization

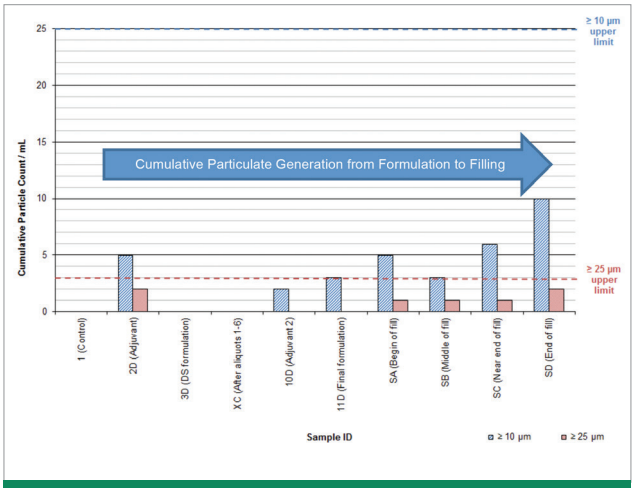

Figure 5: Cumulative Particulate Counts of Formulation and Filling Processes by Light Obscuration as Compared with USP<788> Particle Count Limits – After Optimization

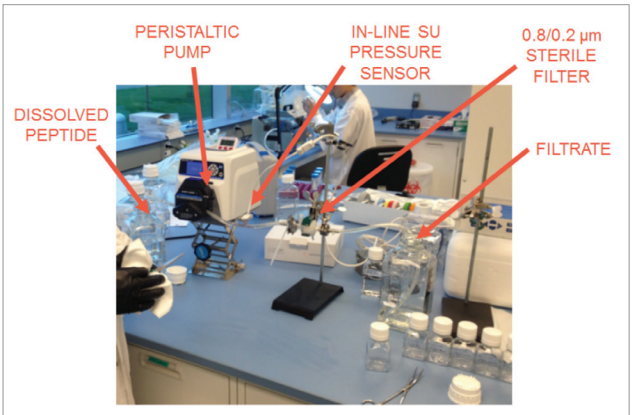

Figure 6: Small Scale Sterile Filtration of a Cationic Peptide by Constant Flow using a 0.8/0.2 µm Filter with in-line Pendotech SU pressure sensor measuring the pressure drop across the filter.

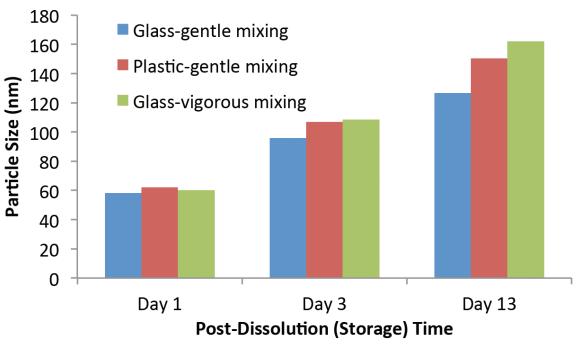

Figure 7: Mean Particulate Sizes Post-Dissolution of a Cationic Peptide (Pre-Filtration) by Dynamic Light Scattering

A Difficult Filtration

In this case, a cationic peptide, vaccine ingredient requires sterile filtration as it cannot be terminally sterilized. The old process involved transferring the ingredient through a sterile filter using a high pressure maximum of 29 PSI or 2 bar. A new process was needed as:

- the intended current GMP facility had no qualified pressure tanks or systems,
- higher pressure applications are, generally, not suitable for single-use, and
- no pressure-rated, steel tanks were available for use.

In addition, there are specific safety precautions that must be taken into account when using a system under pressure. If it can be avoided, perhaps a single-use application would benefit for multiple purposes.

Method

A strategic testing approach was taken with QbD principles applied: such as testing potential critical quality attributes that were impacted by process parameters applied from the peptide dissolution to sterile filtration stages. These attributes included particle size and peptide concentration. In-process controls tested included pressure, product sterility and bioburden. However, for the purposes of the optimization stages, the last two were tested after the process was defined and these test methods were available and/or qualified.

Constant Flow Filtration

The first efforts were to sterile filter the solution by applying constant pump flow through various filter membranes and flow rates at small scale (see Figure 2 for process setup). These included membranes that were recommended to be evaluated as they were neutral or slightly positive to prevent product build-up of the cationic solution on the surfaces of the membrane. Unfortunately, the dissolved material formed long filamentous networks with large clumps aggregating on filter membranes and clogging the filters easily. The pressure reading across the filters, using an in-line pressure sensor, was >20 PSI. Product recovery concentration (by Reverse-Phase High Performance Liquid Chromatography) was reduced to approximately 57-75% and volume recovery was <50% when constant flow filtration was applied across the various membranes. Still above an acceptable amount, the pressure change was reduced when the product concentration was diluted by 33% prior to filtration. It could not be diluted further due to the required final concentration that needed to be obtained. Both pre-filter membranes built into sterile filter capsules as well as independent pre-filters of various porosities were tried with little success. These low recoveries and the fact that the pressure change was higher than what was feasible for the lab's typical SU sterile filtration manifolds, indicates that something in the process needs to change.

Sonication

The next hypothesis was to determine if ultrasonic vibration by sonication could fragment the polypeptide, reducing the particle size and thereby allowing it to pass through a filter more easily. Ideally, this was a technique that could be applied if a single-use, closed system container was used as it can be designed as non-invasive.

This method was tried on the peptide material post-dissolution, taking measurements after several durations of applied sonication. The outcome was that there was no notable impact observed by sonication; no particle size reduction by sonication.

Dissolution

Analysis was performed on the material after gentle and vigorous dissolution methods were tried. In addition, the

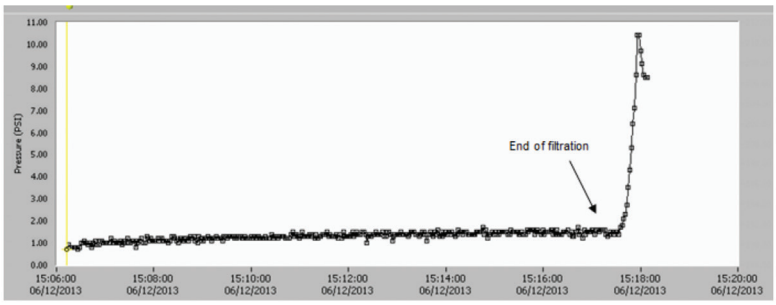

Figure 8: Filtration pressure change of <2PSI using an in-line pressure sensor and monitor. A volume of 0.8L cationic peptide solution passed through a 0.8/0.2 µm sterile capsule filter. Pressure during the filtration remained low, below 1.5 psi, and took approximately 15 minutes to complete. Flow rate was 56 mL/min (± 2mL) upstream, 54.2 mL/min (± 6 mL) downstream.

study challenged glass beaker and single-use, plastic spinner flask containers. The stir bar-to-flask dimension ratios were similar for both containers. The material was dissolved into these containers where they were stored for up to 1, 3, and 13 days to measure particle size change over time.

The mean particle sizes by dynamic light scattering (DLS) (Figure 7) demonstrate that neither container material nor method of dissolution had a dramatic impact on the process. After 13 days, the particle size almost doubled; the storage time after dissolution resulted in the largest change. The recommendation was to try sterile filtration by constant flow a second time, but this time solution was filtered within 1 day post-dissolution to avoid aggregation or filamentous networking.

Outcomes

The data suggests that there is no improvement in recovery or particle size for the following variables: if the product was sonicated, container types, or dissolution mixing speed. Average particle size was maintained when the product dissolution occurred within a day and while using a more dilute concentration to pass through the filter. Following this dissolution, if it was dual sterile filtered in-series immediately, both product recovery by RP-HPLC and volume were 100%, and were therefore improved with this process. In addition, Figure 4 shows a pressure change of less than 2 PSI using an in-line SU pressure sensor and sensor monitor. This took less than 15 minutes to complete, indicating the filter was not clogging as it was before. This graph (Figure 8) shows the filtration of 0.8 L cationic peptide solution passing through the second 0.8/0.2 µm sterile capsule filter in series. Flow rate was 56 mL/min (± 2mL) upstream, 54.2 mL/min (± 6 mL) downstream also indicating that the filter was not clogging. This is a case where the product process was modified to accommodate single-use, as filtration could not be implemented with applied pressure, while at the same time, the product results of interest were improved using this new method. Application of a SU process helped to improve the filtration.

Integrating Integrity

Lately, closed system integrity testing has become an easier option for vaccine manufacturers to do non-destructively, in-house and in-line with new equipment readily available on the market. These machines measure pressure or flow changes within a single-use system that is sealed off from atmospheric pressure. Hole sizes typically start in the detection range of approximately 30-100 µm, as claimed by the testing equipment manufacturers. The purpose of the evaluation was to see how easily the instruments could detect a hole in various types of SU manifolds.

In the final formulation and filling stages of manufacturing a parenteral injectable, building additional levels of sterility assurance is suggested as there is often no further opportunity for sterile filtration (typically terminal sterilization is not performed). The United States Pharmacopeia (USP <1207>, 2015 Draft) recommends that product package integrity testing continue through the life cycle of the product. Keeping in mind that the above USP reference is for packaging, a similar recommendation can apply to the in-process vaccine manufacturing stages. Using a leak integrity tester could detect a hole or breach prior to manufacturing thereby reducing the potential for product contamination, batch rejection or patient risk. The new technology is capable of inflating a bag and measuring it for leaks but this can also be done on an entire assembly with tubing, connections and hard-walled containers all in place. The ability to check integrity of a system design and setup in the end-user's lab space or facility without wetting or compromising the single-use assembly can be used as part of development (for example, verifying a prototype assembly is not leaking), validation, for QC inspection, at time of use or as a form of risk mitigation as a result of a risk assessment or quality inspection. It is up to the user to determine where in the product development and use life cycle this testing could be applied as well as the frequency and test acceptance criteria (i.e., smallest hole size and type).

To prove the equipment can detect leaks successfully with the end-user's general assemblies, several configurations, as shown in Figure 9, were tested including 2D and 3D bags with tubing attached, sterile connectors in-line, single-use filling lines and manifolds. Parameters were setup on each of the three units for each assembly type. Leaks were tested anywhere in the system including the

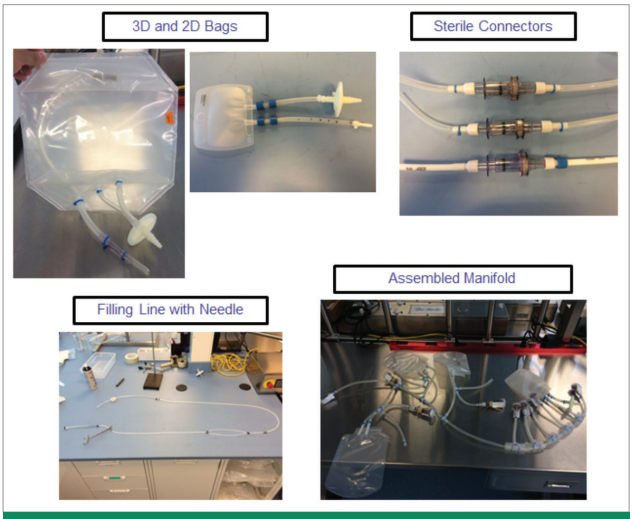

Figure 9: Various Gamma Irradiated, Single-Use, Closed System Components Setup for In-Line Integrity Testing

surfaces of the bags and tubing, bag seams, and junctions at the connection or end points.

Method

The connection to any of the main processing lines to the three brands of integrity testing units was done with an adapter. This either included a built-in sterile filter on the equipment or a separate component that mates by sterile connection to the main-line of the assembly to be tested. This adapter can be single-use and pre-sterile itself but must be designed accordingly (may need to be a custom part to have the correct mating end). The test pressure range for these systems depends on the item being tested, size/length of assembly and other setup conditions but can vary within 3-500 mbar depending on the bag sizes (i.e., 50mL – 1000L) and depending on the unit. A combination of non-defected and defected samples were tested.

A limitation of this form of detection is that anywhere the single-use system has an open or vented line, such as in the case of air/gas vent filters and vented peel-away strips on sterile connectors (or opening in the case of a system with open lines), the lines need to be clamped off at those locations. This creates a limitation in that anywhere downstream of these clamps, integrity is not verified by this equipment.

Some difficulty was had creating the holes or openings in the assemblies to test worst-case scenarios. The integrity testing units have detection leak sensitivity sizes ranging from 30-100 µm, depending on the unit and testing conditions, as claimed by the manufacturers. The bags were sent to an offsite mechanics shop to laser-bore 30 µm holes in plastic. However this task was met with great difficulty in that the plastic in the bags melted and distorted (which seems like an obvious outcome in hindsight!), making it difficult to create fine and measurable positive control holes in the bag material. As a mitigation, two ways of testing the holes were done instead:

- *Pre-calibrated defect* - A calibrated orifice of 100 µm was provided by one of the integrity testing machine manufactures that can be affixed to the unit to use as a control.
- *Manual defect* - Another form of defect was making holes using needles with defined outer diameters as controls.
- In both defect types, the 30 µm hole was not achievable. The other testing machine manufacturers have pre-calibrated orifices that go

Test Samples	Unit A	Unit B	Unit C
3D 5L Bag (no leak)	No leak	No leak	No leak
3D 5L Bag with 290 µm puncture	Leak caught	Leak caught	Leak caught
2D 50 mL bag (no leak)	No leak	No leak	No leak
2D 50 mL bag with 290 µm puncture	Leak caught	Leak caught	Leak caught
Manifold containing 1 L bags (no leak)	No leak	No leak	No leak
Manifold containing 1 L bags with 290 µm puncture	Leak caught	Leak caught	Leak caught
Manifold containing 100 mL bags (no leak)	No leak	No leak	No leak
Manifold containing 100 mL bags with 290 µm puncture	Leak caught	Leak caught	Leak caught
Connectors fully connected (no leak)	No leak	No leak	No leak
Connectors 1/2 connected	Leak caught	Leak caught	Leak caught
Connectors 3/4 connected	Leak caught	Leak caught	Leak caught
Filling line (no leak)	No leak	No leak	No leak
Filling line with 290 µm puncture	Leak uncaught	Leak uncaught	Leak uncaught
Filling line with 390 µm puncture	Leak uncaught	Leak uncaught	Leak uncaught
Calibrated orifice 100 µm	N/Ap.	N/Ap.	Leak uncaught

Table 2: Summary of Leak Integrity Tester Results from Units A, B and C

down to the 30 µm range, however, these were not available at the time the units were tested. However, there are suppliers who have been identified who offer the use of a calibrated orifice.

Outcomes

In the cases of the manifolds, 2D bags, 3D bags, varying bag sizes and connectors, all three testing units generated a pass against the failed positive controls of these units (Table 2). The testing time ranged from less than 10 minutes up to above 30 minutes depending on the unit and the assembly. However, in the case of the filling line, all three units could not detect the defects. The narrow tubing on a sterile, single-use filling line was punctured, making a deliberate slit using a needle with a nominal outer diameter of 290 µm. However, upon testing, the opening did not get detected. Another hole was created using a needle with a nominal outer diameter of 390 µm and the same outcome happened. As the tubing was silicone, the slit that was cut open in the tubing was not detected. It resealed on itself causing the unit to pass. It is hypothesized that the narrow filling line silicone tubing had properties that allowed for reseal of the punctures.

The outcomes of exploring the use of these new integrity testers with typical small-scale, single-use formulation and filling manifolds can be summarized. One must consider where these integrity testers will be most useful. The test time of larger assemblies can be >10 minutes and in some cases much longer. If this is done on every assembly just prior to use, it will add a significant time to the production time in which a batch is executed. Perhaps with a more continuous manufacturing approach in addition to optimization and validation of the process parameters of the unit (i.e., pressure, flow rates), this could be mitigated. Certainly, these units could be made valuable during development and validation activities for testing single-use assembly designs.

Not all types of holes are easy for the units to detect for leaks: larger slits in narrow internal diameter, thick-walled elastomeric tubing, for example, were not detected. In such cases, the slits may "self-seal". In one case, one of the units could not detect the pre-calibrated defect hole of 100 µm. This indicates more optimization is needed of the parameter set points and ranges to ensure the calibration is functional. It is recommended to use the calibrated orifices (slits and round shapes) when calibrating, qualifying or developing testing parameters for a new assembly. Ideally they should come with the purchased equipment.

Different assemblies, parts, materials and sizes will have different process parameters. These need to be optimized and validated for effective use of the integrity units.

Conclusion

Vaccine final formulation and filling processes are optimized and tested to ensure expected, and sometimes better, product performance and process outputs in SU. In the case of particulates, a single-use application should be mapped, sampled and tested to find weak points where higher particulate levels may require modifications and optimization of the process. In the case of filtration, specific product attributes and process outputs need to be measured to meet acceptable levels of recovery, which were improved by applying some process changes and including a single-use process. In addition, in-line SU PAT technologies such as pressure sensors can facilitate detection of product clogging. In the case of leak integrity, there are now testing units on the market that are designed to measure gross leaks in a complete, closed SU assembly. This enables better process monitoring and control of such systems where there were no methods for verifying in-line in the past. The case studies showed that there are situations where the initial vaccine process components, equipment, and processing parameters may need to be changed to accommodate a closed system, single-use process. In the end, it is the user's responsibility to test or have tested the complete assembly in the intended facility using intended or worst-case parameters to build confidence in a process moving into a cGMP manufacturing environment. With these types of contained, controlled and monitored systems, perhaps SU will one-day lead to a manufacturing process that requires no cleanroom at all.

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