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SPOTLIGHT

METHODOLOGY PAPER

A cell therapy media fill protocol for validation of aseptic processing of cord blood

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We present the design of a media fill study protocol using sterile growth medium in place of cord blood for validation of aseptic processing. Growth media are pre-qualified for ability to support growth of relevant microorganisms as visualized in media turbidity assays. Annual completion of media fills are required for all active processing staff to verify use of proper aseptic techniques, executed under 'worst-case' conditions stressing the system. Dynamic environmental and personnel monitoring is included to detect actual contamination risks during the media fills. After processing, all simulated products and controls are incubated and examined for media turbidity. The acceptable failure rate (i.e. observation of turbidity) is defined as zero (0%). All media fills were completed without any failures. Personnel monitoring showed presence of known microbes. These findings demonstrate that the inherent risk for introduction of bio-contaminants, expected to be present during processing, is adequately controlled at CCBC for production of HPC, Cord Blood.

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INTRODUCTION

Aseptic processing is defined in FDA guidance documents as any procedure that is accomplished while minimizing the possibility of microbial contamination [1]. This typically requires sterile or aseptic technique including gloving and gowning, and performance inside the classified environments indicated for all or part of the procedures. Aseptic process validation are studies, also called 'media fills' or 'media fill studies', which simulate actual processing steps, usually with culture medium in place of processing reagents and materials. A successful validation qualifies operators as using proper technique during aseptic operation of critical equipment and confirms that the processing facility has adequate environmental controls to produce sterile products.

Standardized media fill protocols and methodologies have been presented in regulatory guidance for manufacture of small molecule drugs [1]. Driven by the unique requirements for isolation and preservation or enrichment of the desired biological cell-type or cell-related activity [2,3], manufacturing protocols for cellular therapy products are subject to considerable technical diversity. Consequently, demonstration of proper aseptic processing of different cell products via media fills requires customized, distinctive media fill protocols [4–6]. Here we report on the design and execution of a media fill protocol for demonstration of aseptic processing of cord blood, approved by FDA to support the manufacture of HPC, Cord Blood under license [7]. As such, our experience can provide insight into the nuances and expectations for media fill protocols for growing assortments of cellular therapeutics under development towards regulatory approval in the industry.

METHODS

Materials

Cord blood collection bag sets are from Pall (New York, NY). Trypticase Soy Broth (TSB) Growth Media, and qualified TSB in syringes are purchased from QI Medical, Inc. AXP cord blood processing bags and AXP processing system are from Thermogenesis. Irradiated Tryptic Soy Agar (TSA) settle plates and irradiated Tryptic Soy Agar with Lecithin Polysorbate-80 (TSALP) contact plates, and BioBalls strains (*Escherichia coli*, *Aspergillus brasiliensis*, *Candida albicans*) are from BioMerieux.

Turbidity assay, controls & medium qualification

To avoid contaminating the processing facility, these procedures should be performed in designated areas, physically separated from active manufacturing locations. Prior to each annual media fill, 2 or 3 TSB lots were requested from different vendors for lot qualification and acceptance for media fills. For each lot to be qualified, three negative control bags, i.e. collection bags that will not be processed, were prepared by aseptic transfer of 100 mL medium into each bag. To create positive controls, 100 mL qualified medium was aseptically added into each bag and inoculated with 100 CFU USP organisms (BioBall® MultiShot 550 system solutions from BioMerieux Industries). Growth of organisms was facilitated by incubation in a temperature-controlled incubator for 7 days at 30–35°C, followed by 7 days at 20–25°C. Microbial growth was assessed by culture media becoming turbid, in contrast with the enduring clarity of negative control solutions. All positive, and suspected positive, test samples are investigated by plating, sub-culturing and speciation.

To be qualified for use in media fills, test media needed to demonstrate visual clarity and sterility of the solution, with absence of any turbidity or particles, upon receipt and after incubation for 7 days at 30–35°C, and 7 days at 20–25°C. Growth promotion ability was demonstrated by medium turbidity after inoculation with the minimum panel of test organisms and incubation for 7 days at 30–35°C and 7 days at 20–25°C, with

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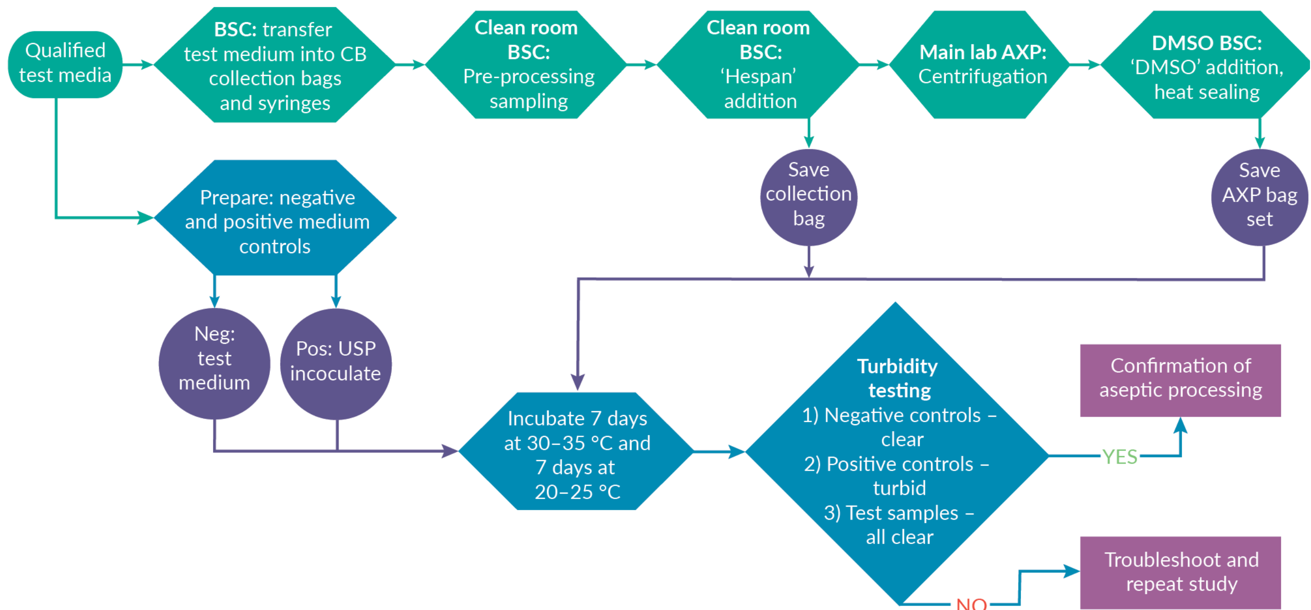
FIGURE 1

Media fill study protocol for processing of cord blood.



Validation of aseptic processing of cord blood is performed via simulation of all technical procedures and equipment under standard operating procedures in their assigned environments, using **(1)** sterile TSB media pre-qualified for ability to support growth of contaminants. **(2)** The test media are aseptically transferred to cord blood collection bags and syringes in a biosafety cabinet, and incubated prior to use to confirm sterility, i.e. lack of turbidity. Processing steps performed inside clean room housed biosafety cabinets include **(3)** acquisition of a pre-processing tests sample, and **(4)** addition of hetastarch. Media fill bags are transferred to main lab for **(5)** AXP system processing via centrifugation. The media fill simulation is completed by **(6)** DMSO addition inside a dedicated biosafety cabinet, followed by heat sealing of retention segments. Media fill bags are not subjected to freezing. **(7)** The collection and AXP bag-sets are saved for microbial growth testing, in parallel with **(8)** control media bags that are either untreated (negative control) or inoculated with test organisms (positive controls). **(9)** Incubation temperatures and times are based on regulatory guidance for optimal growth conditions for microbial test panels including bacterial, fungal and mold species. **(10)** Growth is analyzed via turbidity testing of the media in control bags and processing bags and tubing. **(11)** Confirmation of aseptic processing is defined as absence of media turbidity in negative control and all processing bags systems, and observation of media turbidity in positive control bags.

confirmation of the expected growing organism by speciation.

Processing system & simulation using TSB media

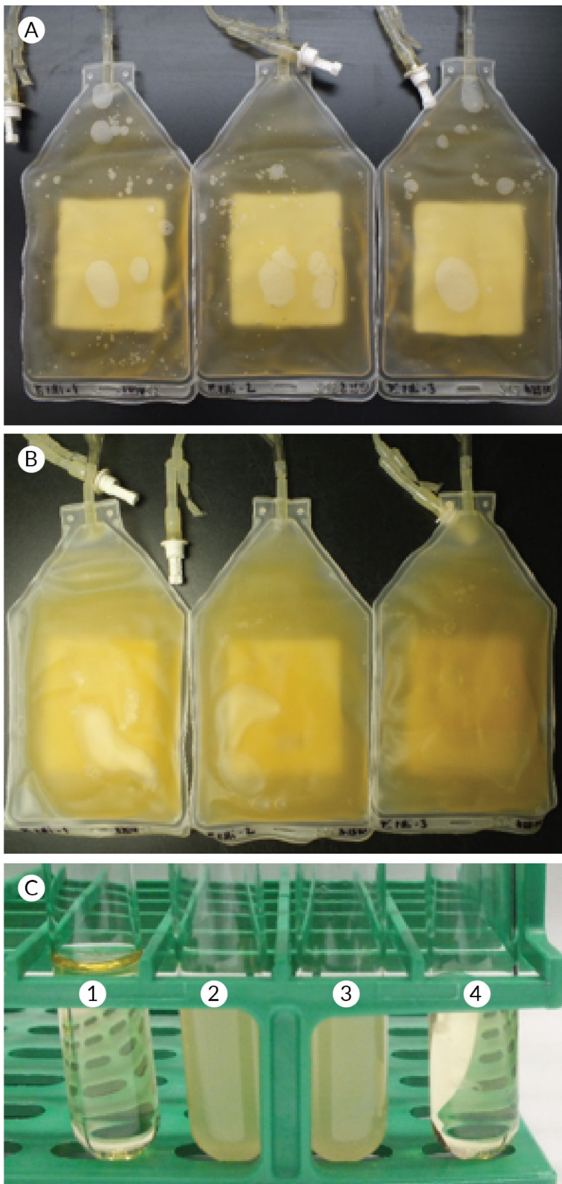
Routine cord blood processing uses the AXP semi-automated, closed blood separation system from ThermoGenesis for partial red blood cell and plasma depletion, and harvest of a nucleated cell population containing the hematopoietic and progenitor cells into a fixed volume. CCBC uses HESPAN® (6% hetastarch in 0.9% sodium chloride injection) to increase stem cell recovery. DMSO and Dextran are added at 10% and 1% final concentration, respectively. During media fill simulations, TSB was used instead of cord blood, hetastarch, and DMSO. AXP centrifugation was performed using a customized centrifugation program designed to achieve a final volume of TSB volume of 20.0 to 22.0 mL in the 5/20 mL freezing bags, the target for cord blood processing. The technical media fill simulations were completed by creation of heat sealed segments after the 'DMSO' addition step. Media fills do not mimic the actual freezing steps. For growth assessment, all processing system elements containing, or exposed to TSB, were collected and incubated for 7 days at 30–35°C and 7 days at 20–25°C.

Environmental monitoring

For dynamic monitoring of viable air particles settle plates were used. The lids were removed

FIGURE 2

Turbidity assay for media qualification.



(A) For qualification for use in media fills, lots of test media are transferred to cord blood collection bags. Before incubation, media should appear clear. **(B)** After inoculation with challenge organisms, such as *E. coli* shown in this example, media should demonstrate visual turbidity. **(C)** After incubation, where needed, turbidity can be further analyzed by transfer of medium samples into clear test tubes. **(1)** Un-inoculated media appear clear, whereas samples from bags inoculated with **(2)** *E. coli*, **(3)** fungus, or **(4)** mold display media turbidity. Bacterial and fungal growth will be evident as uniform turbidity across test sample media. Turbidity of mold cultures may appear less obvious due to tendency for growth in clusters, inside or on top of liquids. Mold cultures may need to be manually dispersed by gentle shaking for more uniform visual turbidity inspection or for sample acquisition for speciation.

to expose the settle plates to the air while placed on a flat surface as close as possible to the critical area of processing activity within the biosafety cabinets. The maximum sample time was 4 hours to avoid the TSA drying out, which could prevent microbial growth. For each batch of settle plates used, negative controls consisting of unexposed plates were incubated along with test plates. Dynamic personnel monitoring was performed using contact ('touch') plates on the dominant hand before and after critical processing activities. Pre-activity samples were taken prior to carrying out any cleaning or tidying operations, to ensure that gloves were dry and free of any disinfectant that might create false negative test results. As sampling technique, the lid of the contact/touch plate was lifted and held by the opposite hand to the dominant hand being tested. The agar surface was touched with the tips of all fingers, followed by the thumb using the gap on the plate within the fingertip impressions. Firm and even pressure was applied to avoid damage to the agar surface. As negative controls, unexposed contact plates were incubated alongside with test samples. All test and control plates were incubated at 30–35°C for 2–5 days, followed by an additional 5–7 days at 20–25°C.

Expected/acceptable results

The acceptable contamination rate is zero (0%) for each annual media fill study. All positive controls, i.e. test media inoculated with microorganisms, must show turbidity. In case of a failure, i.e. any positive test result, the media fill experiment is repeated following investigation to establish the failure root cause and implement appropriate CAPAs to prevent similar occurrences in the future. Positive test results must be investigated by plating, sub-culturing and speciation by a qualified vendor to determine possible contamination sources.

RESULTS

CCBC experience

Media fill studies were first performed at CCBC as an initial qualification of the

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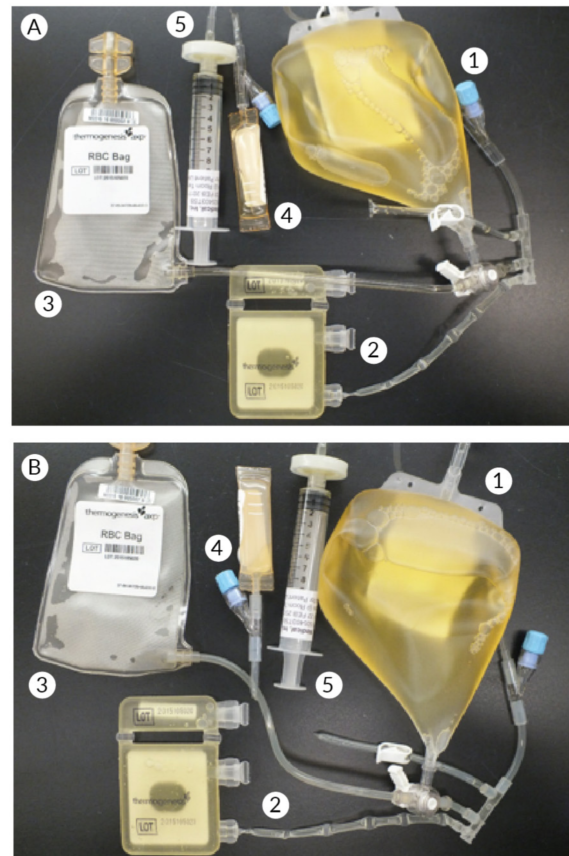
aseptic cord blood processing procedures in 2015, followed by annual revalidations thereafter. Tryptic soy broth (TSB) medium was used in place of cord blood and processing reagents such as hetastarch and DMSO, for simulations of cord blood processing in the sterile, closed system AXP system (Figure 1). All used TSB lots employed in media fills had been pre-qualified in preceding studies for ability to support growth of relevant test organisms by incubation for 7 days at 30–35°C and 7 days at 20–25°C (Figure 1). Microbial growth was assessed by turbidity testing of the media in collection bags during pre-qualification (Figure 2) and in AXP system processing bags and tubing in the media fills (Figure 3). To date CCBC has performed 6 annual studies under the same protocol, including 49 media fills, performed by a total 15 different processing technicians (Table 1). All media fills were completed successfully, with no observation of turbidity (0% failure rate). In all cases, the TSB medium remained clear in all features of the AXP processing bag sets, including the plasma bag, RBC bag, sample pillow and the 5 mL/20 mL freezing bags, the 'DMSO' syringe and bag set tubing (see Figure 3). Each media fill study included tests controls (see Figure 1). All negative controls (uninoculated bags) remained clear, whereas positive control bags, whereas positive control bags (inoculated with microorganisms) showed turbidity after incubation. Speciation of test samples taken from the positive control bags confirmed the identity of the BioBall microorganisms used for inoculation. In all, this demonstrates continued, adequate execution of proper aseptic technique by all processors at CCBC.

To assess the actual risk of introduction of bio-contaminants during processing, dynamic environmental monitoring and operator sampling was performed. Settle plates were present during processing in the Class 100 hoods during processing and contact plate (also referred to as 'touch plate') samples were taken during simulation of the critical processing steps of test sample retrieval, hetastarch addition and DMSO addition (Figure 1, steps 3–5). In all cases, no growth was observed on settle plates (Table 1), demonstrating that the air-quality was as expected and provided no significant contamination risk during processing. In all media fill studies combined, between 3 and 14% of the touch plates showed microbial growth of 2–10 colonies (Table 1). These frequencies were similar to the 6–10% baseline as established by routine personnel monitoring at CCBC (not shown). Positive touch plates were associated

FIGURE 3

Turbidity assessment of bag sets after simulated processing with growth media.



Representative images of an AXP bag set with test media after processing in media fills, **(A)** before, and **(B)** after incubation for microbial growth. Bag set parts include, **(1)** processing (plasma) bag set with attached tubing for connection to cord blood collection bag (not shown in image), **(2)** freezing bag set containing 20 and 5 mL compartments and associated tube segments created after processing by heat sealing, **(3)** red blood cell collection bag used during processing for RBC and volume reduction. Also analyzed are **(4)** AXP bag set sample pouch, and **(5)** the syringe that contained test media used to mimic DMSO addition. After processing and incubation, the media in all processing bag set parts remained clear **(B)**, unchanged from media clarity prior incubation **(A)**.

with various processors over the years (Table 1), without any obvious trending during the media fills. Identified species included a limited set of organisms, mainly gram positive bacteria, all of which had been observed previously in the processing facility via routine environmental monitoring. These findings posed no specific concerns. In all, the results in Table 1 underscore that the media fills at CCBC were performed under conditions reflective of the actual risk due to presence of microbes in the processing environment.

DISCUSSION

Regulatory approval of clinical manufacturing protocols for cellular therapeutics requires demonstration of microbiological control to prevent introduction of contaminants during processing [1]. Here we report on a media fill protocol for validation of aseptic processing of cord blood at CCBC, mirroring the FDA approved protocol for manufacture of licensed HPC, Cord Blood [7]. The central principle is detection of growth of potential contaminants introduced in the TSB test media used during processing simulations, via the simple concept of turbidity assessment. Certain elements of the media fill protocol are driven by the specifics of the product, the methodology used and the scale and complexity of processing. As discussed in Table 2, other media fill principles are more broadly amenable to other cell therapy products, such as integration with environmental monitoring and line clearance procedures.

To facilitate informative turbidity assessment, different TSB test media lots need to be qualified for clearness of the solution and ability to support microbial growth within the containers and devices used during processing (Table 2). Specific panels of well-characterized microbial test organisms (BioBall system) need to be modelled after the baseline for presence of viable contaminants established in environmental and personnel monitoring programs. The FDA approved panel utilized at CCBC include the aerobic bacterium *Escherichia coli*, the mold *Aspergillus brasiliensis*, and *Candida albicans* representing yeast. The test panel microbes should only be used as controls for turbidity assessment, but not in the actual media fill activity, a common misconception. Test microbes should only be handled in designated and controlled laboratory outside of areas of active cell therapy processing activity.

TABLE 1

Five years' experience at CCBC.

Year	# Media fills	# Processors	Failure rate (%)	Positive settle plates	Positive touch plates	# Processors with positive touch plates	Total # of colonies	Detected organisms
2015	15	9	0	0	7/90 (8%)	5	10	Bacillus, Brevibacillus, Corynebacterium, Leifsonia, Micrococcus, Staphylococcus
2016	7	7	0	0	2/42 (5%)	2	2	Micrococcus, Staphylococcus
2017	9	9	0	0	6/54 (11%)	6	9	Micrococcus, Staphylococcus
2018	6	6	0	0	2/36 (6%)	2	4	Micrococcus, Penicillium
2019	7	7	0	0	6/42 (14%)	4	8	Bacillus, Staphylococcus
2020	5	5	0	0	1/30 (3%)	1	2	Micrococcus, Staphylococcus
Total	49	15	0	0	3–14%			

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TABLE 2

Items and expectations for cell therapy media fill protocols.

Items	Relevance and expectations
Turbidity assay	Provides a simple test method applicable to complex, closed system methodologies frequently used in cell therapy production. Requires standard incubation protocols supporting growth of various micro-organisms (e.g. 3–7 days at 30–35°C and 3–7 days at 20–25°C). Visual turbidity evaluation may be supplemented with OD280 measurements. Turbid solutions should be sampled for speciation to confirm the identity of the growing organism(s)
Test media qualification	Select TSB lots with demonstrated ability to support growth of relevant test organisms within the configuration of the containers and (closed) systems used for cell collection and processing
Test organisms	Use well-characterized test microbes (e.g. BioBalls) that represent classes of organisms observed in the processing areas as per environment monitoring programs. Microbes should only be used as controls in turbidity assays, not in the actual media fills. They should only be handled in designated and controlled laboratory outside of areas of active processing activity
Simulation of all processing steps	Media fills need to cover all critical procedures and associated locations involved in manufacturing, particularly any steps that access the product (or media) inside the closed system, e.g. for sampling or addition of processing reagents. Syringes or other containers with pre-qualified TSB lots are often commercially available, or may need to be custom prepared and qualified prior to use in media fill experiments
Testing frequency/ sample size/failure rates	Annually, scheduled over 3 or more consecutive days to facilitate testing by all active staff within the same period. A minimum of 5 media fills annually, or 1 media fill performed by each technician, whichever is more. Expected failure rate is 0%. This has been acceptable for small scale production, e.g. 1,000 products annually, as it applies to public cord blood banking. Other metrics may apply to different cell therapy platforms
Worst-case scenarios	Execute media fills under conditions that stress the system, such as crowding processing locations, slower (or increased) execution of individual processing steps as they might increase risk or extend the risk window for contaminations
Environmental/ personnel monitoring	EM/PM activity needs to be integrated in media fill protocols to identify actual contamination threats, and to confirm worst-case scenario testing
Line clearance	The use and potential spillage of TSB media and test organisms provide obvious contamination risk in the processing spaces. Media fills need to include stringent line clearance procedures to prevent inadvertent increased contamination risk after completion of media fill studies

Turbidity assessment is a convenient, yet non-quantitative, means to track contamination in transparent vessels or cell therapy culture systems, such as the AXP cord blood processing bag system described here. Where needed, incase of non-transparent or opaque culture or vessel configurations, test TSB media need to be sampled after incubation for optical inspection in clear test tubes, as per example shown in Figure 2C. Where needed, these same samples can also be subjected to semi-quantitative spectrophotometric determination (e.g. absorbance measurement at 280 nm).

A basic expectation is that media fills need to cover all critical technical procedures of a manufacturing process and be performed in the associated manufacturing locations and designated environments. Of particular importance are the steps in which the product (or media) inside the closed system is accessed, e.g. for acquisition of in-process test samples or addition of processing reagents. Standard processing reagents such as DMSO may inhibit microbial growth, and their presence could create false-negative outcomes, defeating the purpose of media fill studies. As an alternative, syringes or bags with pre-qualified TSB lots should be used. These are often commercially available. If not, these media fill simulation components will need to be custom prepared and qualified prior to use in actual validations.

Another expectation for media fill studies is performance under 'worst-case scenarios' that stress environmental conditions and the execution of technical procedures by operators. In our experience, a most easily implemented stress-factor is to 'crowd' the processing locations. For this, staff is asked to perform

processing steps as a group, remaining present at the specific processing location (e.g. standing near or sitting at the biosafety cabinet in use,) while colleagues perform their activity, and alternating positions in a reasonable controlled manner to change airflow (e.g. within the clean room environment). Another system stressor that can be used is implementing the maximum acceptable time windows within or between procedures. For example, if the overall standard process is expected to be completed between 4–6 hours, to stipulate in the media fill protocol completion by 6 hours. This can be ensured in different ways, for example if step 2 needs to be initiated within 1–2 hours of completion of step 1, to implement the maximum 2 hour window for the media fill study. These situations serve to mimic circumstances with maximized risk for environmental fluctuations and/or operator distraction under conditions that are still acceptable as per manufacturing protocol.

Testing frequency, sample size and acceptable failure rates are important items that have to be defined for different cell therapy platforms on a case by case basis. CCBC performs annual validations, scheduled over 3 or more consecutive days to facilitate re-qualification of all active staff within the same period. A minimum of 5 media fills are performed annually, or 1 media fill performed by each technician, whichever is more. Expected failure rate is 0%. These parameters have been acceptable for public cord blood banking, with relative small production scales, e.g. 1,000 products annually, using small manufacturing teams. Larger scale or more complex manufacturing platforms involving multiple processing or expansion components, or larger production teams may be required to complete media fills at higher frequency (e.g. quarterly or more) and with larger sample sizes. Acceptable failures, those attributed to processing technique, should be 0%. Any failure should be investigated for root-cause and trigger a repeat study prior to the next scheduled periodic media fill, if feasible.

Finally, all media fill designs contain risk for spillage of TSB media and test organisms, and as such create unintended contamination opportunities in the processing spaces. Therefore, all media fill protocols need to include stringent line clearance procedures to prevent inadvertent cross-contamination during routine procedures upon completion of all media fill activities.

REFERENCES

1. Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/sterile-drug-products-produced-aseptic-processing-current-good-manufacturing-practice
2. Lipsitz YY, Timmins NE, Zandstra PW. Quality cell therapy manufacturing by design. *Nat. Biotechnol.* 2016; 34(4): 393–400.
3. Bravery CA, Carmen J, Fong T *et al.* Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry. *Cytotherapy* 2013; 15(1): 9–19.
4. Serra M, Roseti L, Bassi A. Media Fill for Validation of a Good Manufacturing Practice-Compliant Cell Production Process. In: Turksen K. (eds) Stem Cells and Good Manufacturing Practices. *Methods Mol. Biol.* 2014; vol 1283. Humana Press, New York, NY.
5. Sigward E, Fourgeaud M, Vazquez R, Guerrault-Moro MN, Brossard D, Crauste-Manciet S. Aseptic simulation test challenged with microorganisms for validation of pharmacy operators. *Am. J. Health Syst. Pharm.* 2012; 69(14): 1218–24.
6. Urbano N, Modoni S, Schillaci O. Media Fill Test for validation of autologous leukocytes separation and labelling by (99m)Tc-HmPAO. *Nucl. Med. Biol.* 2013; 40(1): 104–8.
7. CLEVECORD (HPC Cord Blood): https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/clevecord-hpc-cord-blood

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Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

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