Single-use workflow for cell culture medium preparation

This application note demonstrates the performance of a single-use workflow for reconstitution of powdered cell culture medium. Medium hydration was performed using Xcellerex™ XDM Quad Mixing System 1000 L. Hydrated medium was filtered using ReadyToProcess™ disposable filter assemblies. ReadyMate™ disposable aseptic connectors were used for connection of the reconstitution and filtration steps into a closed system. Growth performance of prepared medium was evaluated. The results were found to be comparable with medium hydrated using conventional reusable equipment.

Introduction

Cell culture powder medium is the preferred format over liquid media for transportation and storage purposes due to compact volume and favorable stability. Prior to use, powder medium is often hydrated on site, using reusable mixing vessels. The hydration process is labor-intensive and requires cleaning and cleaning validation. Reuse of hydration vessels imposes risk of cross-contamination. To overcome these challenges, we present a disposable solution for hydration of powdered cell culture medium with advantages of ease of use and proven scalability.

Reconstitution of powdered cell culture medium was conducted using an XDM Quad Mixing System 1000 L followed by sterile filtration using ReadyToProcess disposable bioprocessing assemblies. Prepared medium was evaluated with analytical and cell growth performance assays against a medium sample hydrated using conventional equipment. In addition, scalability of the process was investigated.

Materials and methods

Hydration of cell culture medium

ActiCHO™ Powder Base CD medium was hydrated according to recommended protocol using the XDM Quad Mixing System 1000 L. An XDM-1000 Basic single-use bag was installed in place and filled with reverse osmosis/deionized (RO/DI) water to 96% of the target volume. Stirring was turned on and kept at 200 RPM in counter clockwise direction during the hydration process. Powder medium was added to a concentration of 22.36 g/L to the mixing bag through the 3 inch top port. After 60 min of mixing, sodium hydroxide solution (10 N) was added to a concentration of 3.25 mL/L through the liquid addition line. After another 30 min of mixing, sodium bicarbonate powder was added to a concentration of 1.8 g/L through the 3 inch top port followed by an additional 60 min of mixing. Subsequently, liquid volume was adjusted to target by adding RO/DI water. A final mixing of 15 min was conducted before completion. Total time for the hydration process was about 3 h. A small-scale medium hydration using a 1 L glass beaker was conducted in parallel to serve as control.

Medium samples were dispensed from the sampling line. Osmolality and pH were measured using an Advanced™ Model 3300 Micro-Osmometer (Advanced Instruments, Norwood, MA) and a Pinnacle M545 Benchtop pH Meter (Cole-Parmer, Vernon Hills, IL), respectively. Medium samples were also analyzed for glucose, glutamate, sodium, and potassium using a BioProfile™ 400 analyzer (Nova Biomedical).

Filtration of cell culture medium

Upon completion of hydration process, approximately 1 L of liquid was dispensed from the sampling line and sterilized using a 0.2 µm Stericup™ filter (EMD Millipore, Billerica, MA). Remaining liquid in the XDM mixing system was filtered through a filtration train as depicted in Figure 1. Filtration was driven by a Watson-Marlow™ 520 peristaltic pump (Watson-Marlow, Wilmington, MA). A two-step filtration strategy, consisting of an ULTA™ Pure HC 0.6/0.2 µm filter capsule and an ULTA Pure MR 0.1 µm filter capsule, was applied. Presterilized parts of the filtration train were aseptically connected using ReadyMate connectors. Multiple ReadyCircuit™ single-use bags were connected to downstream of the ULTA Pure MR filter via a ReadyCircuit Jumper T Manifold.

During filtration, the initial 5 L permeate served as filter flush and was collected in a ReadyCircuit bag designated for waste. The permeate flow was subsequently switched to another bag to start filling of prepared liquid medium. Medium filling moved to the next bag when reaching the current bag's capacity. Control medium hydrated in 1 L beaker was filtered using a Stericup filter.

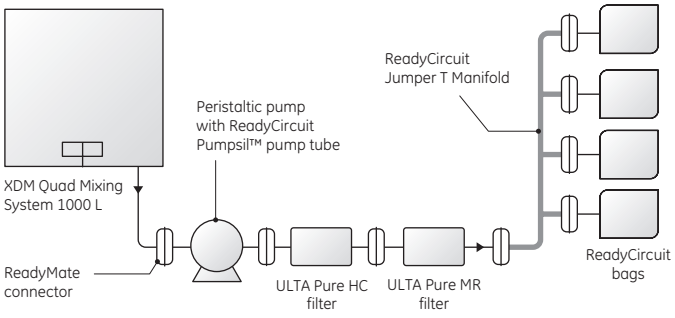

Fig 1. Diagram of single-use cell culture medium preparation workflow.

Cell growth performance assay

Cell culture medium samples were assessed in a cell growth performance assay. The model cell line was a CHO DG44 derived cell line (licensed from Cellca GmbH) expressing a monoclonal antibody. Cells were cultivated in each medium sample for two passages prior to being seeded for a batch cell culture assay. Target seeding density was 3×10^5 cells/mL and initial culture volume was 40 mL in 125 mL shake flasks. Cell cultures were placed inside a Multitron™ incubator (Infors AG, Bottmingen, Switzerland) set at 120 RPM, 36°C, and 5% CO_2. Cell culture samples were taken immediately post inoculation and daily, starting from day 3, for determination of viable cell density and viability by a Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter, Brea, CA). Cell cultures were terminated on day 7.

Results

Liquid samples collected from the XDM mixing system and 1 L beaker hydrations were subjected to measurements of pH and osmolality as well as analyses of medium component levels. The results are shown in Table 1. All measured values are similar between the two samples with differences less than 4%, suggesting that the XDM mixing system can provide adequate mixing for hydrating powder medium as do conventional small-scale mixing equipment.

Table 1. Analytical results of liquid samples hydrated in XDM-1000 and in 1 L beaker

	Hydration vessel		
Analysis	**1 L beaker**	**XDM mixing system**	**Difference %**
pH	7.39	7.33	- 0.8%
Osmolality (mOsm/kg)	300	308	2.7%
Glucose (g/L)	6.28	6.04	- 3.8%
Glutamate (g/L)	2.62	2.67	1.9%
Na^+ (mM)	103	106	2.9%
K^+ (mM)	9.6	9.9	3.1%

A set of four cell culture medium samples were tested in cell growth performance assays (Table 2). Sample 1 was hydrated in the control 1 L glass beaker and filtered with a Stericup filter. Sample 2 was hydrated in the XDM mixing system and filtered with a Stericup filter. Samples 3 and 4 were both hydrated in the XDM mixing system and filtered through the ULTA Pure filtration train, with the former being collected at the start of medium filling and the latter at the time of reaching theoretical filter capacity.

Table 2. List of medium samples tested in cell growth performance assay

Sample ID	Hydration vessel	Filtration	Note
1	1 L beaker	Stericup	n/a
2	XDM mixing system		1 L sample from XDM mixing system
3	XDM mixing system	ULTA Pure HC and ULTA Pure MR	Sampled at the beginning of medium filling
4	XDM mixing system		Sampled upon reaching filter theoretical capacity

Growth curves are shown in Figure 2. Cells in all test medium samples exhibited excellent growth, reaching high concentration of 10×10^6 cells/mL by day 4 and maintaining viability greater than 95% through day 6. Table 3 summarizes representative growth indicators, such as peak viable cell density (VCD), integral viable cell density (IVCD), and cell population doubling time. The results of medium samples from mixing using the XDM system (Samples 3 and 4) are comparable with the control sample from the 1 L beaker (Sample 1), indicating successful medium preparation in this single-use workflow.

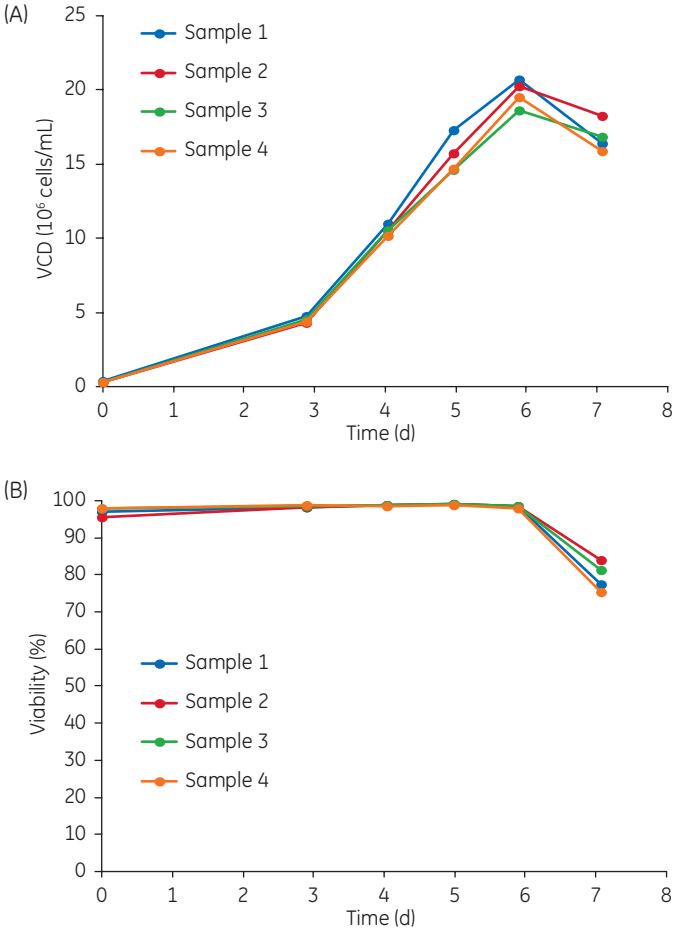

Fig 2. Cell growth performance assay: (A) cell growth and (B) viability. Samples 1 through 4 in legend correspond to sample IDs in Table 2.

Table 3. Cell growth characteristics in medium samples

Sample ID	Peak VCD (10^6 cells/mL)	IVCD (10^6 cells/mL/d)	Cell population doubling time (h)
1	20.7	69.0	18.4
2	20.2	66.6	17.3
3	18.6	63.6	18.1
4	19.5	63.4	18.3

Discussions

One important consideration regarding cell culture medium hydration is to provide adequate mixing for complete solubilizing of powder medium, while avoiding over-mixing. Mixing systems differing drastically in configuration (e.g., impeller size and type, vessel size, shape, and aspect ratio) between scales can result in differences in mixing characteristics. This could create challenges for scale-up and transfer of the medium hydration processes. Application of mixers with standardized design and configuration across scales provides a solution to this challenge. The single-use XDM mixer family, with standardized configurations for seamless scalability, offers the capability for medium reconstitution at a wide range of scales, from 20 to 2500 L. In this study, the XDM Quad Mixing System, with working volumes up to 1000 L, was used for large-scale cell culture medium hydration.

Cell culture medium is commonly sterilized using a 0.2 µm filter. A developing trend is implementing a two-step filtration scheme by adding a 0.1 µm filtration step downstream of the 0.2 µm filter. Benefits of a two-step filtration scheme include the capability of removing mycoplasma and other small microorganisms that pass through the 0.2 µm filtration. Due to the possibility of non-specific binding of trace nutrients by the 0.1 µm filter, evaluation shall be performed to confirm the equivalence of medium performance before adapting a two-step filtration scheme. In this study, medium filtered by ULTA Pure two-step filtration train performed similarly to medium filtered by a 0.2 µm Stericup filter, suggesting the ULTA Pure two-step filtration would not compromise medium performance. Moreover, medium samples collected at the beginning and end of the filtration step both exhibited comparable performance as the control medium, confirming full filtration capacity being achieved.

Conclusion

A process for large-scale cell culture medium preparation from powder was developed using a single-use mixing system coupled with a two-step filtration train. The process inherits multiple advantages of single-use techniques, such as reduced risk of cross-contamination, decreased cleaning and cleaning validation costs, and elimination of equipment sterilization step. Moreover, the two-step filtration train added an extra layer of confidence for medium sterility. Easy-to-use aseptic connectors made tube welding redundant. Implementation of this workflow could realize significant cost- and time-savings in cell culture medium preparation.

Ordering information

Product	Description	Product code
XDM-S Quad Mixing System	1000 L	29054861
XDM-1000 Basic	1000 L	888-0167-C
ULTA Pure HC 0.6/0.2 µm	2 inch filter capsule	12410093
ULTA Pure MR 0.1 µm	2 inch filter capsule	12410294
ReadyMate DAC	aseptic connector	28936688
ReadyCircuit bag	5 L hanging/pillow bag	12410221
ReadyCircuit bag	200 L 3D bag	12410209
ReadyCircuit Jumper T Manifold	with four ports of 6 in of AdvantaPure™ reinforced silicone 0.5 in (13 mm) i.d. tubing terminating with ReadyMate connectors	12410179

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