
# User Requirement Specification (URS) for Single Use Bioreactor (SUB) Systems for perfusion cell cultures

VERSION 1

February 19, 2024

Revision History

| Revision Number | Date | Description of Changes |
|---|---|---|
| 0 | December 20, 2023 | Original Version |
| 1 | February 19 | Final Version |

NIMBL

Table of Contents

# 1 Purpose and Scope

The purpose of this User Requirements Specification (URS) is to specify a Single Use Bioreactor (SUB) system for perfusion cell cultures in accordance with its technical requirements and applicable current local and regulatory, environmental, health, and safety, and automation standards.

The intent is to define the critical aspects (CAs) and critical process parameters (CPPs) on which the system qualification will be based. This document also details performance requirements and various design criteria for the equipment to be used in the pharmaceutical environment under cGMP conditions. However, it is not the intent of this document to detail all mechanical, electrical and control requirements. The vendor shall supply all subordinate components necessary to meet the performance requirements established herein. Should the vendor find it necessary to deviate from the specific design and performance requirements detailed in this document, the vendor shall clearly state all the deviations in the proposal and give the reason(s) for each deviation.

The Single Use Bioreactor (SUB) systems for perfusion cell cultures will be referred to as "system" in this document. The system will be used in a continuous cell culture process for manufacture of biopharmaceutical products (e.g. monoclonal antibody production (Mab)). The system will operate continuously for thirty (30) days. During this time a constant stream of cell culture media and feed(s) will be supplied to the system. The system will used to perform several process phases, including a/ installation of the single use components, b/ filling with initial growth media, c/ inoculation with cells, d/ a cell growth phase to reach working cell density, e/ a production phase where the product is continuously harvested through the cell retention device and optionally a cell culture bleed is applied to maintain cell density at a fixed level, and f/ a termination phase where the bioreactor contents are harvested and the subsequently the single use components are removed and discarded. The equipment will be installed in a cGMP environment, specifically in non-hazardous Grade C or Grade D clean room with temperature control (15-25°C).

The system(s) will be used for 500-Liter, 2,000-Liter, or 4,000-Liter perfusion processes over a range of titers. Multiple single-use flow paths will be required to cover this range.

The single-use, product contact flow path assembly is specified in the requirements section of this document. Cell culture media and Feed product bags, final product collection bags, cell culture bleed collection bags, buffer solution totes and Cell Retention Device (CRD) will be connected to this system to operate the perfusion cell culture steps. The requirements for the feed and intermediate product bags, buffer totes and CRD filters are not within the scope of this URS.

## 2 Area of Application

This URS applies to the following systems:

| Item | Description | Tag Number |
|---|---|---|
| 1 | SUB system (for perfusion) | SUB001 |

## 3 Responsibilities

| Function | Purpose of Signature |
|---|---|
| Local User | I am signing on behalf of the user and confirm that this document accurately reflects the technical user requirements. |
| Project Engineering | I am signing/authorizing this document and agree that the technologies specified in this document are correct and in line with current technical concepts and that each requirement is specific and measurable for requirements affecting Product Quality. |
| Automation | I am authorizing this document and agree that the automation requirements specified in this document are correct and in line with current technical concepts. |
| Health, Safety and Environment | I am authorizing this document and agree that the requirements specified in this document are in line with current health, safety and environmental standards. |
| Quality | I am signing on behalf on the Quality Unit and confirm that the content of this document is compliant with relevant internal and external cGMP standards. |

## 4 Process

The Single Use Bioreactor (SUB) system for perfusion cell cultures will be used for the cell culture and production of the clarified harvest intermediate. The cells cultured in the bioreactor are engineered to excrete the protein of interest, typically Mab, into the surrounding cell culture media. Subsequently the cell retention device, a cross flow filter, is used to retain the cells in the bioreactor and pass the protein of interest into the clarified harvest stream. Subsequently the clarified harvest stream is used as input for the subsequent purification or downstream processing operations.

Figure 1 shows a typical set-up of a perfusion bioreactor with ingoing flows of (cell culture) media, feeds, base and antifoam. And outgoing flows of (cell free) clarified harvest and (cell containing) cell bleed.  The cell retention device connected (CRD) to the bioreactor, contains a cross flow filter that retains the cells in the bioreactor and passes the product of interest, typically being Mab.

Figure 2 shows a typical example of a perfusion process in a SUB, where after inoculation, in the growth phase the cell density increases to the required high cell density under increasing perfusion flow to match the nutritional requirements of the cells. Subsequently, typically at a constant and high cell density achieved by cell bleeding, the product of interested as excreted by the cells is collected in, and further processed from the clarified harvest container.

*Figure 1. Example of a Single Use perfusion bioreactor set-up with cell bleed included*

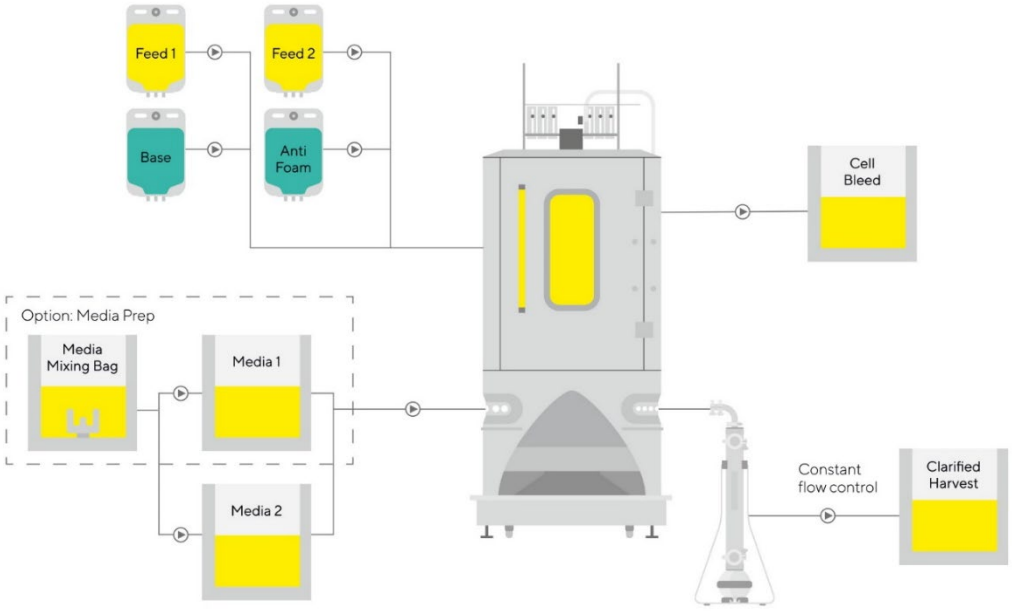
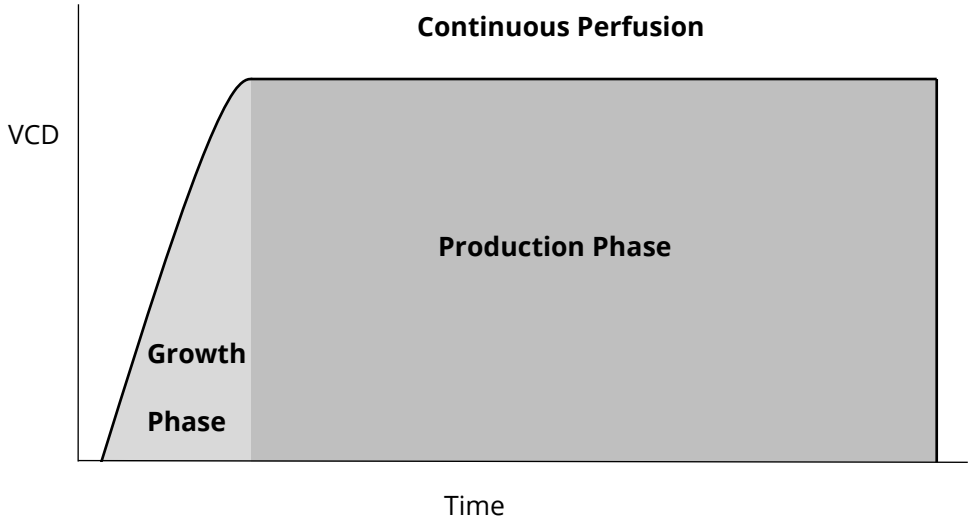



*Figure 2. Example of Phases in perfusion process.*



The process steps shall consist of the following:
1. Installation of the single use components SUB, CRD, Transfer sets, single use bags for liquid inputs and outputs
2. Pre-use rinsing of the CRD according to supplier's specifications
3. Taring of balances and filling of the SUB with initial growth media
4. Calibration of probes and preparation for inoculation
5. Inoculation with cells from the seed train
6. Cell growth phase to reach working cell density (e.g. up to 125 mln cells/mL) with increasing media and feed perfusion to match the cellular nutritional needs
7. Production phase where the product is continuously harvested through the cell retention device and optionally a cell culture bleed is applied to maintain cell density at a fixed level, and with the option to change to enriched production media during the production phase
8.Termination phase where the bioreactor contents are harvested and the subsequently the single use components are removed and discarded

# 5 Requirements

## 5.1 Product Quality Critical Assessment

An assessment has been performed to determine which requirements listed in this specification are critical to product quality. Where a requirement is deemed to be product quality critical, "Yes" is stated. If a requirement has been determined - as non-critical to product quality, one of the following justifications is provided as its rationale:

(1) **Health, safety, and environment critical** (for personnel and/or equipment only).
(2) **Business critical** (productivity, operations, process efficiency).
(3) **Technical requirement** with indirect or no impact on product quality, patient safety, and CGMP data integrity.
(4) **Non-product contact** (applies to equipment that is not in direct contact with the product).
(5) **Critical** but quality critical requirement is covered by another requirement in this URS. Reference is provided to the critical requirement.

The justifications (1 to 5) above are applicable for the entire document. At least one justification shall be stated per requirement.

## 5.2 General Requirements

Reference the General Equipment URS for details.

## 5.3 Functional Requirements

| ID | Requirement | Product Quality Critical (Yes/No) | Comments / Questions |
|---|---|---|---|
| | **General SUB bag characteristics** | | |
| FNC-01 | The different bag sizes should have a maximal working volume of [L]<br>- 500<br>- 2000<br>- 4000 (Or 2 x 2000L) | N | 2, 3 |
| FNC-02 | The turn-down ratio of the bags shall be at least 1:5 | N | 2, 3 |
| FNC-03 | The bag must withstand an operational pressure of at least 50 mbar (on the headspace) plus the hydrostatic pressure created by the liquid height in the Bioreactor | N | 1 |
| FNC-04 | The bag must be leak-testable (at point of use), such as pressure decay methods | N | 1 |
| FNC-05 | The bag should be able to operate for at least 30 days | N | 2 |
| | **Mixing characteristics** | | |
| FNC-06 | The stirrer design should allow a mixing time of <45 seconds | N | 3 |
| FNC-07 | The stirrer design allows for gentle (acceptable shear rate) stirring of mammalian cells, with a shear rate of <3500/sec while meeting all other agitation requirements. | Y | |
| FNC-08 | The stirrer design must allow a power input that enables KlA of 14 to 100 1/h | N | 3 |
| FNC-09 | The stirrer design must withstand densities of up to 5 cp | N | 3 |
| | **Gassing characteristics** | | |
| FNC-10 | The bag must allow gassing with up to four different gasses | N | 3 |
| FNC-11 | The bag must allow for headspace gassing | N | 3 |
| FNC-12 | The bag must allow for submersed gassing via one or more sparger(s). | N | 3 |
| FNC-13 | The sparger(s) must allow maintaining cell culture densities of at least 125 mln c/mL, while:<br>1) generating minimal foaming with sparger gas exit velocity of <15 m/s.<br>2) Reduce aerosol particle generation to lower load on exhaust line | Y | |
| FNC-14 | The bag must allow an aeration rate of up to 0.2 VVM | Y | |
| FNC-15 | Clogging of exhaust filter must be minimized. When clogging occurs back-up exhaust filters shall be available. Automatic switch over is preferred but is optional. | Y | |

| | | | |
|---|---|---|---|
| FNC-16 | The system shall prevent exhaust filter blocking in high humidity conditions. | N | 1 |
| | **Liquid flow characteristics** | | |
| FNC-17 | The bag must at least be equipped with the following tube lines<br>- inoculation<br>- sampling<br>- pH adjustment<br>- Main media-in line<br>- feed medium A<br>- feed medium B<br>- bleed line<br>- Cell Retention Device connection(s)<br>- Harvest line | Y | |
| FNC-18 | The media-in and CLD permeate lines must allow a perfusion rate up to at least 2 VVD | Y | |
| FNC-19 | All tubes must be weldable and sealable AND have AseptiQuik genderless sterile connectors | Y | |
| FNC-20 | Design must allow cell bleed through aseptic connections | Y | |
| FNC-21 | Design must allow repetitive, sterile sampling | Y | |
| FNC-22 | Tubing lines must be compatible with pumping over extended use time | Y | |
| FNC-23 | Upon harvesting less than 2% of the bioreactor volume should remain in the bioreactor bag | N | 2 |
| | **Cell retention characteristics** | | |
| FNC-24 | Preferred cell retention devices are tangential filtration based, e.g. TFF or ATF | N | 3 |
| FNC-25 | The cell retention device(s) must be able to support mammalian cultures of at least 125 mln c/mL at 2 VVD perfusion flow | Y | |
| FNC-26 | The cell retention device should allow sterile swapping during operation to allow sterile installation of back-up replacement CRD during operation | Y | |
| FNC-27 | At least 2 cell retention devices should be able to be connected to the bioreactor and operated in parallel at scales of 2000L or greater | N | 2 |
| FNC-28 | The different bag sizes must be connectable to ATF and TFF module via ports, that vary by SUB size:<br>500L - 0.75"<br>2000L: 1"<br>4000L: 1.5" | Y | |
| FNC-29 | The hardware system must allow the operation of inbuilt and external ATF or TFF circulation pumps/systems | Y | |
| | **Temperature control loop** | | |
| FNC-30 | Bag design must allow for online temperature measurement | Y | |
| FNC-31 | pT100 probes must operate for at least 30 days within a working range of 0.05 % accuracy | Y | |

| | | | |
|---|---|---|---|
| FNC-32 | Heating of the full bioreactor content from 20°C to 37°C shall be possible in 4 h | Y | |
| FNC-33 | Cooling of the full bioreactor content from 37°C to 8°C shall be possible in 4 h | Y | |
| | **Pressure control loop** | | |
| FNC-34 | Measurement of the headspace pressure for overpressure safety is needed | Y | |
| FNC-35 | Headspace pressure sensors shall work over the required range. Range to be recipe variables, with typical values of -0.1 - 0.7 bar. Accuracy of pressure sensors must be sufficient to insure safe operation of the system at 37 °C | Y | |
| FNC-36 | Pressure in the sparger lines shall be monitored and alarmed on high pressure to detect sparger fouling/blockage | Y | |
| | **Agitation control loop** | | |
| FNC-37 | RPM should be measured within 5 rpm accuracy | Y | |
| FNC-37 | RPMs allowing tip speeds of at least 1.8 m/s should be possible. | Y | |
| | **Level control loop** | | |
| FNC-38 | Level should be measured within 1 % accuracy | Y | |
| FNC-39 | Level shall be controlled through filling via the medium-in pump and/or through emptying via the perfusion and flow pump(s) such that a stable level and culture is maintained. | Y | |
| | **pH control loop** | | |
| FNC-40 | Two pH probes required for redundancy. Built-in SU pH probes are preferred if accuracy and drift requirements for 30 days of operation can be met (Optional) | N | 2, 3 |
| FNC-41 | pH probe design must be of aseptic design to support 30 day cell culture process. | Y | |
| FNC-42 | pH probes must allow working range of 6-8 w/ 0.1 % accuracy at 37 °C | Y | |
| FNC-43 | Downward pH control shall be through CO2 sparging | Y | |
| FNC-44 | Upward pH control shall be through Base addition | Y | |
| | **DO control loop** | | |
| FNC-45 | Two DO probes are required for redundancy. Built-in SU DO probes are preferred (optional). | N | 2, 3 |
| FNC-46 | DO probe design must be of aseptic design to support 30 day cell culture process. | Y | |
| FNC-47 | DO probes must allow working range of 5%-100% w/ 5 % accuracy at 37 °C | Y | |

| | | | |
|---|---|---|---|
| FNC-48 | DO control shall allow advanced cascade. DO control should support setpoint control (+/- 15%) at cell densities of up to at least 125 mln cells/mL (with approximate KlA of 65 1/h).<br>Preferabley DO control can be adjusted by choosing optimal sparger types, impeller types and implementing optimal stirrer speeds. air and oxygen gas flows.<br>Preferably pCO2 can be independently controlled by choosing (separate) optimal sparger types, and CO2, air and oxygen gas flows. | Y | |
| | **Perfusion flow control loop** | | |
| FNC-49 | Perfusion flow measurement must allow working range of 0 - 2.5 VVD with 2 % flowrate accuracy at 37 °C | Y | |
| | **Cell bleed control loop** | | |
| FNC-50 | Cell bleed flow measurement must allow working range of 0.05 - 0.15VVD with 2 % accuracy at 37 °C | Y | |
| FNC-51 | Bleed flow should be controlled via the bleed pump | N | 3, 5 |
| | **Additional feed control loop(s)** | | |
| FNC-52 | Perfusion feed flow must allow working range of 0 - 2.5 VVD with 2 % accuracy at 37 °C | Y | |
| | **Viable cell control loop** | | |
| FNC-53 | Bag(s) design shall use online sensors for cell density measurements | N | 2, 3 |
| FNC-54 | Viable cell density (VCD) control required by cell bleed flow control to maintain constant VCD | Y | |
| | **Miscellaneous control loops** | | |
| FNC-55 | System design shall allow (as an option) for off-gas measurements of O2 and CO2, to determine OUR, CER and RQ | N | 2 |
| FNC-56 | Bag(s) design shall allow (as an option) the use of online sensors for measurement of ciritical metabolites, e.g. Raman, NovaFlex, HPLC | N | 2 |
| FNC-57 | The system shall allow automatic adjustment of perfusion rates based on process events (time, cell densities, ...) | Y | |

## 5.4 Cleaning, Sanitization and Steaming in Place Requirements

See General Equipment URS for specifications.

## 5.5 Utility Requirements

| ID | Requirement | Product Quality Critical (Yes/No) | Comments / Questions |
|---|---|---|---|
| UTL-01 | System shall be able to connect to the following utilities:<br>Instrument air (0 to 7 barg)<br>Water / glycol temperature control system (0 to 6 barg)<br><br>System to have pressure reducing valve to keep water pressure below safe operating pressure of system. | N | 3 |
| UTL-02 | The gas inlets of the system shall be equipped with pneumatic quick connects. | N | 3 |
| UTL-03 | Flow path to system inputs and from system outputs shall be (multi-use) hoses for the following fluids:<br><br>-Instrument air<br>-Heating/cooling fluid | N | 3 |
| UTL-04 | The system shall be operable with safety gloves (one or two pairs). | N | 1 |
| UTL-05 | Vendor shall provide utility connection information, steady state and peak consumption demands. | N | 3 |

## 5.6 Automation Requirements

See General Equipment URS for specifications.

## 5.7 Metrology Requirements

See General Equipment URS for specifications.

## 5.8 Electrical Requirements

See General Equipment URS for specifications.

## 5.9 Health, Safety, and Environment

See General Equipment URS for specifications.

### 5.10 Maintenance requirements

See General Equipment URS for specifications.

### 5.11 Flexibility

See General Equipment URS for specifications.

### 5.12 Scope of required documentation

See General Equipment URS for specifications.

## 6 Abbreviations & Acronyms

| Abbreviation / Acronym | Description |
|---|---|
| URS | User Requirement Specification |
| SUB | Single Use Bioreactor |
| CRD | Cell Retention Device |
| CGMP | Current Good Manufacturing Practice |
| CRD | Cell Retention Device |
| Mab | Monoclonal Antibody |

## 7 Attachments and References

### a. Attachments

| # | Title | Doc. No. |
|---|---|---|
| | | |
| | | |

### b. References

| # | Title | Doc. No. |
|---|---|---|
| 1 | | N/A |
| 2 | | N/A |