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1.0 Purpose

This procedure describes the operation procedures for the NBS CelliGen PRO 150L Bioreactor.

2.0 Scope

This SOP applies to all Biopharmaceutical Development Program (BDP) personnel operating the NBS CelliGen PRO 150L Bioreactor.

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Biopharmaceutical Development Program (BDP), has the authority to define this procedure.

3.2 Production personnel are responsible for training on this procedure and for documenting training to Biopharmaceutical Quality Assurance (BQA).

3.3 Production personnel are responsible for the implementation of this procedure.

3.4 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 NBS CelliGen PRO 150L Bioreactor

4.2 4” Vent filter, BDP PN 22134

4.3 Mini Emflon Filter, BDP PN 20487

4.4 pH 4 buffer, BDP PN 30107

4.5 pH 7 buffer, BDP PN 30108

4.6 Water for Injection (WFI)

4.7 Nitrogen Gas

5.0 Procedure Guidelines

5.1 Use the Batch Production Record (BPR) and equipment logbook to document this procedure.

6.0 Procedure

6.1 Utility Startup

NOTE: Refer to **Attachment 1** for the exact valve position for all bioreactor processes. Also, verify that all utility lines are hooked up to their corresponding supply/return piping before turning on any of the utilities to the vessel(s).

6.1.1 Electric

6.1.1.1 Power on the vessel.

6.1.1.2 Login to the vessel Panelview.

6.1.1.3 In the MAIN screen, press the ACTIVATE button to make the vessel operational.

6.1.2 Process Waste

6.1.2.1 Verify that the skid drain fitting is connected to the Biowaste line. Verify closed the CIP return valve and open the ball valve for the Bio-waste system.

6.1.3 Process and Instrument Air

6.1.3.1 Open the pharmaceutical air and compressed gas valves on the utility panel.

6.1.3.2 Open the vessel skid instrument air supply valve **HV8A**. Verify that it is supplying 90+5 PSIG on pressure gauge **PI8A**.

6.1.3.3 Open the vessel skid process air valve **HV1A**. Verify that it is supplying 30+5 PSIG on pressure gauge **PI1B**.

6.1.4 Medium Pressure Steam

6.1.4.1 Open the medium plant steam supply and return valves at the utility panel.

6.1.4.2 Open the vessel skid valve **HV6A**, utility steam. The steam pressure should stabilize to 40 $\pm$ 5 PSIG as read on the pressure gauge **PI6A**.

6.1.5 Pure Steam

6.1.5.1 Verify that the steam process bypass elbow is attached to the tri-clamp fitting labeled “steam”. If installed correctly the signal indicator light should be lit.

6.1.5.2 Open the pure steam valve on the utility panel.

6.1.5.3 Open the vessel skid process steam valve **HV9A**. Verify that the supply pressure stabilizes to 35 $\pm$ 5 PSIG as read on the pressure gauge **PI9A**.

6.1.6 Chilled Water

6.1.6.1 Open the process chilled water supply and return valves on the utility panel.

6.1.6.2 Open the vessel skid facility water valve HV4A and the water return valve **FCV5B**.

6.1.7 Oxygen Gas (if utilized for processing)

6.1.7.1 Open the oxygen gas valve on the utility panel.

6.1.7.2 Open the vessel skid oxygen valve **HV10A.**

6.1.7.3 Verify that the pressure gauge **PI10A** read 30+5 PSIG.

6.1.8 CO_2 Gas (if utilized during processing)

6.1.8.1 Open the CO_2 gas valve on the utility panel

6.1.8.2 Open the vessel skid CO_2 valve **HV10B**.

6.1.8.3 Verify that the pressure gauge **PI10B** reads 30+5 PSIG.

6.1.9 N_2 Gas (if utilized during processing)

6.1.9.1 Open the N_2 gas valve on the utility panel.

6.1.9.2 Open the vessel skid N_2 valve **HV10C**.

6.1.9.3 Verify that the pressure gauge **PI10C** reads 30+5 PSIG.

6.1.10 Agitator Seal Pressure

6.1.10.1 Verify the agitator return seal pressure is 35+5 PSIG on pressure gauge **PI24A**.

6.1.11 Exhaust Vent

6.1.11.1 Open/verify that the vent valve at the utility panel is open.

6.1.12 pH Probe Installation

6.1.12.1 Consult the BPR or appropriate logbook for the serial numbers of the product specific probes for use. (A back-up pH probe may be installed per the BPR.)

6.1.12.2 Inspect the pH probe for visual damage such as cracks partially hidden by the housing. Confirm that the pH cable is connected to the probe and the transmitter.

6.1.12.3 On the top DO/pH transmitter, press the MENU function key; choose Calibrate by using the arrow keys, then press the ENTER key.

6.1.12.4 Select S1 (pH), BufferCal, Auto, Start Auto Cal.

6.1.12.5 Rinse the probe with WFI and immerse the probe into pH 7 buffer and press ENTER.

6.1.12.6 After the transmitter stabilizes, the user will be presented with a range of pH values that are close to the Buffer1 value. Select the number that is as close as possible to Buffer1's actual value, press ENTER.

6.1.12.7 Rinse the probe with WFI and immerse the probe in the pH 4 solution; press ENTER.

6.1.12.8 Again, the transmitter will stabilize within a range of pH values that are close to the Buffer2 value. Select the number that is as close as possible to Buffer2's actual value; press ENTER.

6.1.12.9 The transmitter will indicate if the calibration is successful or unsuccessful. If the pH probe will not standardize after two iterations of this procedure, then replace the pH probe and/or cable, and repeat procedure.

6.1.12.10 Press the EXIT key until you return to the Menu Screen.

6.1.12.11 Rinse the probe with WFI water and install into the vessel.

6.1.12.12 Repeat the same process in Steps 6.2.7.2 through 6.2.7.10 for a second pH probe. This time the standardization will be performed on the bottom DO/pH transmitter.

6.1.13 pH Probe Re-standardization

6.1.13.1 On the pH/DO transmitter, press the MENU button. Select the Calibrate option and press ENTER.

6.1.13.2 Select Sensor 1 from the options listed; press ENTER, then proceed to pH and then Standardize in the same fashion.

6.1.13.3 Enter the offline value by pressing the Up/Down and Right/Left arrow buttons. Press ENTER to accept the value entered.

6.1.13.4 Use the EXIT key to return to the pH/DO transmitter's main menu.

6.1.14 Dissolved Oxygen (DO) Probe Installation

6.1.14.1 Consult the BPR or appropriate logbook for serial numbers of DO probes for use.

6.1.14.2 Verify that the membrane is less than six months old and that the probe(s) is/are polarized.

6.1.14.3 Connect or verify connection of the DO cable to the probe and transmitter.

6.1.14.4 On the DO/pH transmitter, press the MENU function key; using the arrow keys choose Calibrate and then press the ENTER key.

6.1.14.5 Place the probe into a test chamber connected to the nitrogen supply on the utility panel. Select S2 (DO), Oxygen, and open the nitrogen supply at a pressure sufficient to purge the atmosphere surrounding the probe of all oxygen. Select Zero Cal and press ENTER. The sensor will begin zeroing. Once finished, the transmitter will return to the Oxygen selection screen where the Air Cal option will be available. Shut off the nitrogen supply.

6.1.14.6 Set the span by allowing the probe to stabilize in air, navigate to Air Cal, and press ENTER. Select Start Calibration and set the atmospheric pressure to 760.0 mm Hg by using the arrow keys. Press ENTER. Once

the probe has stabilized Cal. DONE will display and the user will be returned to the Air Cal screen.

6.1.14.7 Press the EXIT key until you return to the Menu Screen.

6.1.14.8 If the response is slow or unstable, rebuild the DO membrane as described in ***SOP 12118 - Maintenance and Storage of Dissolved Oxygen and pH Probes***.

6.1.14.9 Rinse the probe with WFI water and install it into the vessel.

6.1.14.10 Repeat the same process in Steps 6.2.9.2 through 6.2.9.9 for a second DO probe. This time, the standardization will be performed on the bottom DO/pH transmitter.

6.2 Vessel Preparation

6.2.1 Connect a flexline from the discharge port to the drain.

NOTE: Flush the point of use for at least 30 seconds before use.

6.2.2 Open the vessel drop valve and rinse the interior surfaces of the vessel for a minimum of one (1) minute with WFI.

6.2.3 Verify in the equipment logbook that the vessel was cleaned prior to use per ***SOP 12222 - CIP of the NBS CelliGen Pro 150L Bioreactor.*** Collect a sample per ***SOP 12169 - Rinse Water Sampling for Production Equipment*** from the sample port if required per the MPR.

6.2.4 Allow the vessel to drain completely.

6.2.5 Disconnect the flexline from the discharge port and install a stainless-steel end cap.

6.2.6 Obtain two (2) Pall 4 inch 0.2 µm filters (BDP PN 22134) and one (1) mini Emflon filter (BDP PN 20487). Record the filter information on the BPR or in the equipment logbook if no BPR is used. Install the 4-inch filter in the inlet and exhaust housings, and the mini Emflon in the overlay air filter housing.

6.3 Vessel Pressure Check

6.3.1 Install any other analytical probe(s) as required by the BPR.

6.3.2 Verify all vessel penetrations and valves are shut and secure.

6.3.3 Set Pressure control to 20 psi and set to AUTO.

6.3.4 Set Airflow control to 20 slpm and place in AUTO.

6.3.5 Set the Overlay control to 10 slpm and place in AUTO.

6.3.6 Once pressure builds to 20 ± 5 PSIG, turn off the exhaust valve at the utility panel. Also turn off the Overlay control loop.

6.3.7 Observe vessel pressure for a minimum of five minutes for pressure loss.

6.3.8 Correct any deficiencies before proceeding and note resolution in the equipment logbook.

6.3.9 Depressurize the vessel by setting the Pressure control to 5 psi.

6.3.10 At this time, the Biocommand computer can be attached to the vessel using the NBS Biocommand RS232 communication cable and dongle. Once logged into the system, the user can then start a batch that will collect input data from all of the vessel's control loops.

6.4 Sterilization of Empty Vessel

6.4.1 Open the Growth window by pressing the Growth button at the bottom of the Panelview. Press the Start Batch button to begin a production batch. A batch must be started before the subsequent sterilization cycle can be performed. If the batch is not started, the sterilization cycle will have to be performed again.

6.4.2 Enter loop set points per the BPR for the vessel during Growth phase into each vessel controller. Set all control loops used for Growth phase to AUTO, except for Temperature and Agitation control. Set the Pressure control loop to 10 psi and in AUTO mode along with the 4-GAS to ON. This will help minimize any pressure lost during the cool down from sterilization.

6.4.3 Verify that **PI7A** is reading 20 – 25 psi. If needed, the pressure can be adjusted into the correct range using **PCV7A**.

6.4.4 Access the sterilization options by pressing the STERILIZE control button. Enter the following parameters that will define the length and temperature of the cycle.

6.4.4.1 Drain: 3 Minutes

6.4.4.2 Heat B: 100.0°C

6.4.4.3 Sterilize Temp: 125.0°C

6.4.4.4 Hold: 25 Minutes

6.4.4.5 Cool B: 47.0°C

6.4.4.6 Growth: 37.0°C

6.4.5 Open the vessel drop valve **FCV19** and associated condensate valve **FCV19B**

6.4.6 Open the condensate valves for the sample port/addition ports.

6.4.7 From the STERILIZATION Screen press the START button to activate the SIP process. Next, press START CYCLE to begin the process. Upon initialization the water from the jacket will drain. After the drain period, steam will be injected into the jacket.

6.4.8 See **Attachment 1** Sterilization Valve Matrix for proper valve position during sterilization.

6.5 SIP setpoint: 125.0°C.

6.5.1 When the set point is reached, open the steam valves for the addition and sample ports: **FCV18**, **FCV20**, **FCV26**, **FCV27**, and **FCV28**.

6.5.2 At the Panelview, observe the PHASE TIME timer to confirm sterilization timing.

6.5.3 Within the last five (5) minutes of sterilization, close the vessel drop valve **FCV19** and the associated condensate valve.

6.6 Vessel Cool down: 125°C to 37°C

6.6.1 Close the steam and condensate valve(s) for each addition/sample port and the drop valve: **FCV18**, **FCV20**, **FCV26**, **FCV27**, and **FCV28**. When closing the valve(s), the condensate valve should be closed first.

6.6.2 After the sterilization HOLD time is satisfied as read from the PHASE TIME timer, the vessel will initiate cool down.

6.6.3 Monitor the vessel temperature and pressure during cooling. Confirm that vessel pressure remains < 25 and > 0 psi as read by **PI9C**.

6.6.4 When the cool down is complete verify that the pressure stabilizes to the preset vessel pressure from Step 6.5.1. All other Growth phase setpoints should return to normal as the vessel equilibrates.

6.6.5 Make a logbook entry including: Date, Time, “SIP Complete”, Production Lot Number, and Initials.

6.7 Setting Vessel Parameters

6.7.1 At the Panelview, verify each of the needed control loops are controlling and set to their appropriate mode per the BPR.

6.7.2 Set EXHAUST FILTER HEATER to ON from the CONTROL 3 Screen. Verify that the loop enters Manual Mode.

6.7.3 Alternatively, if an alternate exhaust filter heater is used instead of the integrated NBS filter heater, verify that the heater is powered and displaying a temperature setpoint. Verify that the displayed temperature setpoint is 80°C

6.8 Vessel Batching

6.8.1 Tare the load cells by pressing TARE on the Load Cell Indicator. Note that the vessel must be empty and the jacket full for accurate batching values.

6.8.2 Using a vessel addition port, aseptically add the volume of media per the BPR or Supervisor’s instructions. Record the final load cell reading in the BPR.

6.8.3 At the Panelview, verify each of the needed control loops/alarms are controlling and set to their appropriate mode per the BPR.

6.9 Vessel Inoculation

6.9.1 Pull a sterile BI sample before inoculating the vessel using the sampling procedure outlined in Step 6.11. Refer to the BPR for details.

6.9.2 Verify the growth parameters, control cascades, pump assignments and rate, and period values are set as described in the BPR.

Using the DO/pH transmitter box, span the DO to 99.99% by selecting the MENU Button, Calibrate, Sensor 2, Oxygen, and then In Process Cal. The DO value at this point should be set to +99.99% by using the arrow keys up/down and right/left. Press ENTER when finished and use EXIT to return to the main menu.

6.9.3 Continuing at the DO/pH transmitter, set temperature compensation to automatically read the vessel temperature. This is done by selecting the MENU Button, Calibrate, Oxygen, and Temperature. Scroll down to Sensor 2 temperature compensation. Press ENTER to enable if the option is currently disabled.

6.9.4 Re-standardize the pH probe to match the offline BI value by following the Steps in 6.2.7.12.

6.9.5 Add the inoculum to the vessel using the addition procedure outlined in Section 6.10.

6.9.6 Reset the E.F.T to reflect the start of culture growth.

6.10 Aseptic Additions

6.10.1 Attach the addition/inoculation assembly to an addition port and attach a condensate line directed to a trap.

6.10.2 Open the condensate valve (on addition/inoculation assembly) and corresponding addition steam valve for the port and SIP for a minimum of 15 minutes. (follow temperature guidance in step 6.11.4 and documentation requirements in step 6.11.6)

6.10.3 Close the condensate valve and then the steam valve to maintain positive pressure. Allow the addition/inoculation assembly to cool for a minimum of 5 minutes. (The vessel addition port valve may be opened at this time to facilitate cooling).

6.10.4 Open the valve on the addition/inoculation assembly and the vessel addition port (if not yet opened) to allow the flow of material to enter the vessel.

6.10.5 Pump the material into the vessel using a peristaltic pump. Close the vessel addition port valve when addition is complete and close the valve to the addition/inoculation assembly.

6.10.6 Briefly open the condensate and steam valves to the addition port to purge residual material from the assembly (see **NOTE** after Step 6.11.9).

6.10.7 Close all steam supply and condensate valves and allow the addition/inoculation assembly to cool until it can be safely handled before removal.

6.10.8 Remove the addition assembly and reinstall the condensate line on the addition port.

6.11 Vessel Sampling (Sterile)

6.11.1 Connect the sampling assembly in line between the sample port discharge and the condensate line.

6.11.2 Connect a calibrated temperature sensing unit and corresponding RTD to the spool piece connected to the sample port steam trap. Turn on the temperature sensing unit before proceeding to the SIP of the sample port.

6.11.3 Open the sample port steam supply (**TCV18A**) and condensate valve (on sample assembly).

6.11.4 Verify that the sample port steam trap temperature achieves ≥123.5°C. Once at or above this temperature, the addition port should be allowed to sterilize for ≥15 minutes. The sterilization time must restart if the temperature falls below 123.5°C. If the sample port steam trap never achieves the required temperature or time, contact the Area Supervisor.

6.11.5 Close the condensate valve and then steam valve to maintain positive pressure. Allow the sampling assembly to cool for a minimum of 5 minutes.

6.11.6 Upon successful port sterilization, record an equipment log entry that includes the SIP complete and port valve identification.

6.11.7 Open the sample valve **TCV18**.

6.11.8 The condensate valve may be opened briefly to allow a small amount of process liquid to flow to Biowaste to ensure a representative sample.

6.11.9 Open the sampling assembly valve and collect the sample.

6.11.10 Close the sampling assembly valve and the sample valve **TCV18**.

6.11.11 Open the steam supply (**TCV18A**) and condensate valve briefly to purge residual material from the assembly (see **NOTE** after Step 6.11.9)

6.11.12 Close the steam supply and condensate valve and allow the sampling assembly to cool until it can be safely handled before removing. Re-attach the condensate line or attach another sample assembly.

NOTE: For some projects it may be necessary to perform a complete decon SIP based upon the safety profile of the product. The BPR or Supervisor will provide directive if a full decon SIP is required.

6.12 Vessel Harvest

6.12.1 The vessel includes a removable condensate valve (**FCV19B**) at the vessel drop valve. If a sterile harvest is required, this valve must be removed by disconnecting its sanitary connections.

6.12.2 An autoclaved, sterile, transfer valve should be inserted into the vacant condensate valve location. The transfer valve must include its own valve to divert condensate to Bio-waste.

6.12.3 SIP the vessel drain port for a minimum of 15 minutes by opening steam supply valve **TCV28A** and the condensate valve attached to the harvest assembly. After SIP is complete, close the condensate valve and then steam supply valve to maintain positive pressure.

6.12.4 Connect directly to the harvesting equipment or receiving vessel.

6.12.5 Once the harvest procedure is ready to begin, press Harvest on the Growth screen and open the vessel drain valve **FCV19**.

6.12.6 When harvest is complete, press End Batch at the Growth Screen. Disconnect the harvest line, re-attach the condensate piping, end the batch on Biocommand, and proceed to ***SOP 12222 - CIP of the NBS CelliGen PRO 150L Bioreactor*** (if cleaning the same day of production.)

6.13 Vessel Shutdown

6.13.1 Turn off all utilities opened in Steps 6.1.1 through 6.1.7. This would include both the utility panel, vessel skid utility valves, and electrical disconnect.

7.0 Documentation

7.1 Document the performance of this procedure on the BPR and in the equipment logbook.

8.0 References and Related Documents

SOP 12118 *Maintenance and Storage of Dissolved Oxygen and pH Probes*

SOP 12169 *Rinse Water Sampling for Production Equipment*

SOP 12222 *CIP of the NBS CelliGen PRO 150L Bioreactor*

9.0 Attachments

9.1 **Attachment 1** Sterilization Valve Matrix

Attachment 1

Sterilization Valve Matrix

M1366-0095

CONTROL SYSTEM INSPECTION PROCEDURE

Valve Sequence Table

VALVE TAG	VALVE POSITION	DESCRIPTION	CONTROL VALVE TAG	NOTES	DRAIN	HEAT A	HEAT B	STERIL	COOL A	COOL B	GROWTH
FCV 3A	NO	Exhaust	FY 3A		ON	ON	ON	ON	ON	ON	
TCV 5N	NC	Exhaust Cond.	TY 5N			ON	ON	ON			
FCV 2B	NC	Inlet Air	FY 2B								ON
PCV 7B	NC	Vess. Pressurization	PY 7B						ON	ON	
TCV 5E	NC	Inlet Filter Steam	TY 5E			ON	ON	ON			
TCV 5C	NC	#1 Inlet Filter Cond.	TY 5C			ON	ON	ON			
TCV 5H	NC	Sparger Cond.	TY 5H			ON		OSCIL			
TCV 6A	NC	Jacket Steam	TY 6A			ON	ON	ON-OFF			
TCV 5K	NC	Circulation	TY 5K							ON	ON
TCV 5A	NC	Heat Exchanger	TY 5A							ON	ON
TCV 5B	NC	Water Return	TY 5B						ON	ON***	ON *
TCV 5L	NO	Jacket Block	TY 5L		ON	ON	ON	ON			
TCV 5M	NC	Jacket Cond.	TY 5M		ON	OSCIL	OSCIL	OSCIL			
TCV 5P	NC	#1 Exh. Filter Cond.	TY 5P			ON	ON	ON			
FCV 23A	NC	Sparger	FY 23A				ON	ON-OFF	ON**	ON**	ON
FCV 23B	NC	Ferm. Overlay	FY 23B	Option			OSCIL	OSCIL	ON	ON	
TY 24A	NC	Seal Condensate	TY 24A				OSCIL	OSCIL			
P-5A		Circulation Pump	P 5A							ON	ON
TCV 4A	NC	Condenser Water	TY 4A	Option					ON	ON	ON
TCV 5F	NC	#2 Inlet Filter Cond.	TY 5F	Option		ON	ON	ON			
TCV 5R	NC	#2 Exh. Filter Cond.	TY 5R	Option		ON	ON	ON			
FCV 16A	NC	CIP	FY 16A	Option							
TCV 17A	NC	Glycol Cooling	TY 17A	Option						OFF****	ON
TCV 35A	NC	Overlay Steam	TY 35A	Option		ON	ON	ON			
FCV 35B	NC	Overlay Air	FY 35B	Option							ON
TCV 35C	NC	Overlay Condensate	TY 35C	Option		ON	ON	ON			
FCV 35D	NC	Overlay Vessel	FY 35D	Option			ON	ON-OFF			ON

Notes: * OFF if Glycol option is present
** OFF if Ferm. Overlay option is present
*** OFF if Glycol used to cool during sterilization
**** ON if Glycol used to cool during sterilization

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