TITLE: Operation of the Biospherix Xvivo Closed Incubation System

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A. OBJECTIVE

This document describes the Operation of the Biosperix Xvivo Closed Incubation System in The University of Iowa Dezii Translational Vision Research Group (DTVR).

B. APPLICABILITY

This document applies to all personnel at DTVR.

C. REFERENCES

D-DEZ-PRO-001 Dermal Biopsy Sample Collection
D-DEZ-PRO-002 Plating, Passaging and Freezing FBs from Dermal Biopsy Punch
D-DEZ-PRO-003 Keratinocyte Isolation from Dermal Biopsy Punch
D-DEZ-PRO-004 Generation of iPSCs
D-DEZ-PRO-007 iPSC Culture and Differentiation
D-DEZ-PRO-008 Culturing of iPSC Retinal Pigment Epithelium
D-DEZ-PRO-015 Evaluation of CRISPR-mediated Nonhomologous End-joining (NHEJ) Recombination
D-DEZ-PRO-017 Preparation of Keratinocyte Media
D-DEZ-PRO-020 Transient Lipofection in Adherent Cells
D-DEZ-PRO-022 Clinical Virus Production and Purification
D-DEZ-PRO-035 293FT Cell Culture
D-DEZ-PRO-036 Nucleofection

D. PROCEDURE

1.0 SAFETY

1.1.1 The Xvivo System Safety Considerations

1. The Xvivo System has a variety of safety issues including electrical, gas, pressure, and ventilation. Some minor mechanical safety issues may also arise.
2. See Instruction Manual for a detailed list of all safety considerations.

1.2 SYSTEM MODULES

1.2.1 The Biospherix Unit consist of the following:

1. Buffer Module: Each Buffer Module consists of one or more Buffer chambers. A Buffer chamber is the controlled, closed environment that is used as the entrance and exit for the Xvivo System.
2. Processing Chamber: Used for general processing of cells.

3. Incubation Chamber: The function of the Incubation Chamber is to provide absolute optimal conditions for the long-term culturing of cells in vitro.
4. Incubator Bank Module: This primary function is to control the internal temperature of the Incubation Chambers mounted inside.

1.3 SYSTEM UTILITIES

1.3.1 System Power

1. Each electrical power panel is located behind a clear, protective cover which can only be removed with a tool. In these panels, are mounted power entry sockets, power switches, and a resettable circuit breaker for each individual power circuit.

1.3.2 Electrical Power Up Procedure

1. Ensure that the main power cables are plugged into the receptacles on the Main Power Panel, and that the power source in turned on.
2. Turn on the Main Power Switches at the Main Power Panel(s).
3. Turn on the Control computer and launch the Xvivo control application.
4. Make sure the System Status Bar reads Communication OK. “Communication Lost” will be displayed if any of the Controller Cards are disconnected or not fully functioning.
5. Open the Control Screen for the Processing Chamber and click the “CRAC” toggle button to turn on the “CRAC” unit.
6. Click the “Light” toggle switches to turn ON the Processing Chamber lights, if needed.
7. With the system power functioning properly, System Startup can be initiated.

1.3.3 Electrical Power Down Procedure

1. From the System Control computer, turn off all Xvivo System gas control. Make sure that the “Sample Stream Pumps” are OFF.
2. Click the “Light” toggle switches to turn “OFF” the Processing Chamber work lights, and the Laminar Flow Hood work lights.
3. Click the “CRAC” toggle button to turn “OFF” the “CRAC” unit, the Fan will turn off.
4. Turn off the gas supplies at the regulators.
5. Turn off all vacuum supply at the vacuum source.
6. Remove power from all third-party equipment that is located in any of the Xvivo System Chambers.
7. Turn off the Main Power Switches at the Main Power Panel(s).
8. With all power removed from the Xvivo System, the Control computer can be shut down.

9. If maintenance is going to be performed on the system, the Main Power Cables should be unplugged, and the "BLEED" valves on the back of the Utility Module should be opened to bleed off any remaining gas pressure.

1.3.4 Gas Supply to the Utility Module

1. The main ¼" ID Supply Hoses are connected to the Utility Module as follows:

1.3.4.1.1 The main Nitrogen supply is connected to the NITROGEN barb on the back of the Utility Module.

1.3.4.1.2 The main Oxygen supply is connected to the OXYGEN barb on the back of the Utility Module.

1.3.4.1.3 The main Carbon Dioxide supply is connected to the CARBON DIOXIDE barb on the back of the Utility Module.

1.3.4.1.4 The main Span Mix supply is connected to the SPAN MIX barb on the back of the Utility Module.

1.3.5 Gas Supply Pressure

1. The gas supply pressure used throughout the Xvivo System is maximum 40 p.s.i. The incoming pressure must be regulated to 0-40 PSIG.

1.3.6 Connecting Gas Supplies to the Xvivo

1. The Utility Module is the location where control gas is supplied to the Xvivo. Gas is supplied to the Xvivo system through ¼" ID tubing connected to hose barbs on the back of the Xvivo Unit.
2. The incoming pressure must be regulated to 0-40 psig.
3. The amount of gas used is dependent on the process levels being controlled, and how often and how long the chamber doors are open.

1.3.7 Connecting the Gas Supplies

1. Make sure the regulator is completely closed
2. Make sure the compressed gas source is completely closed
3. Screw the regulator onto the compressed gas source
4. Repeat the first two steps for all compress gas sources

1.3.8 Vacuum Supply

1. Vacuum supply is required for fully sealed Xvivo Systems to provide pressure equalization.
2. The vacuum requirements for the Xvivo System are variable and intermittent. Therefore, when the system is not in use it is recommended to turn off the vacuum supply to prevent damage to the vacuum.

1.4 HUMAN-MACHINE INTERFACE, HMI

1.4.1 Diagram, System Display Screen

1. Click on any of the modules in the diagram to open the Control Screen for that Module.
2. All chambers are labeled, and will become highlighted when touched. Incubator Bank Modules are always adjacent to the Incubation Chambers, and are highlighted separately when touched. "All Control Stopped" will flash over any chamber when control is turned off in that chamber.

1.4.2 System Status Bar

1. Located across the top of the screen, the System Status Bar provides several important system indications.

1.4.2.1.1 User Name – Name of the user currently logged into the system.

1.4.2.1.2 Date and Time – Displays the current date and time.

1.4.2.1.3 Systems Communications Indicator – The field displays Communication OK (green) when all controller cards are properly connected and functioning. Communication Lost (red) will be displayed if any of the controller cards are disconnected or not fully functioning.

1.4.2.1.4 Alarms Indicator – This field will remain green and will display No Alarms when there are not active system alarms. If any alarms are active, the field will flash red and display the number of active alarms until all of the alarms have been normalized.

1.4.2.1.5 About – Displays the version/release date of the currently installed software.

1.4.2.1.6 Administrator Control – Only accessible with proper permission. (See page 1-47 of User Instruction Manual for Administrator Control Screen)

1.4.2.1.7 Change Password – Touch to open the password screen.

1.4.2.1.8 Live Alarms – Touch to open the live alarms.

1.4.2.1.9 Alarm History – Touch to open the alarm history page.

1.4.2.1.10 Shutdown – Click to shutdown the software

1.4.2.1.11 Light ON/Light OFF – Click to toggle Laminar Flow Hood work light on and off.

1.4.3 Administrator Control Screen

1. The Administrator Control Screen is used to set minimum and maximum Process Control Setpoints for the individual chambers. To change a Setpoint Limit, click the "Setpoint" field, and enter the desired range using the "Virtual Keyboard". Once these parameters are entered, Chamber Control will be limited to these settings. These settings will also be displayed on the window bar of the "New Setpoint Value Virtual Keyboard" as (Min S.P. to Max S.P.)

   Note: This screen is only accessible to users with the proper permissions set by the Administrator. A detailed description to this screen is available in the Instruction Manual, page 1-47.

1.4.4 Event Log Screen

1. This screen is used to view System Event history.

1.4.5 Chamber Control Screen

1. Used to control all functions of an individual chamber. Control screens will vary depending on the chamber type and the Process Control options available in the chamber.

   Note: A detailed description of the Chamber Control Screen is available in the Instruction Manual, on page 1-49 to 1-51.

1.4.6 Calibration Control Screen

1. This Control screen is used to calibrate a single sensor. This screen is equipped with all of the controls needed to calibrate the sensor, as well as the Trend Plot Chart to simultaneously view sensor OutPut.

   Note: A detailed description of the Calibration Control Screen is available in the Instruction Manual, on page 1-52 to 1-54.

1.4.7 Tuning Control Screen

1. Tuning adjust the control to be efficient and tight. It's a perfect tune if the control process involves no overshoot, no undershoot, and no oscillation.

   Note: Tuning Control is explained in detail in the Instruction Manual, page 1-55 to 1-59.

1.4.8 Trend Chart

1. Used to easily view Process Control functions in a graph format. The Y axis reflect Process levels and the X axis reflects time.

   Note: Trend Charts are explained in detail in the Instruction Manual, page 1-60 to 1-62.

1.4.9 Export Data

1. The Export Data window is launched by clicking the "Export Data" button on the Trend Chart screen and selecting "Open". All historical process data can be exported for a single chamber, the data is saved to a file that can be opened as a spreadsheet or a text file.

   Note: Export Data is explained in detail in the Instruction Manual, page 1-63.

1.4.10 Alpha Numeric Virtual Keyboard

1. The Alpha Numeric Virtual Keyboard opens whenever a field that can be modified is clicked on, for example, Setpoint. The Alpha Numeric Virtual Keyboard can be used to enter all alpha-numeric characters used for system control using the Control computer mouse. Characters can also be entered using the Control computer keyboard.

Note: Alpha Numeric Virtual Keyboard is explained in detail in the Instruction Manual, page 1-64.

1.4.11 Process Control Operations

1. All Process Control Operations can be initiated from the Chamber Control Screen. All available Process Control Variables are listed in the left column, followed by all the current Process Control information including Process Value, Setpoint, OutPut Up\Down, Mode Status, and Control ON/OFF status. Additional functions can be assessed by clicking the "Tuning" and "Calibration" buttons.

Note: Process Control Operations is explained in detail in the Instruction Manual, page 1-65 to 1-66.

1.4.12 Process Alarms

1.4.12.1 There are six process alarm types

1. Process Deviation Plus: Alarm type will activate if the process variable goes above the current setpoint plus the current Deviation Plus setting.
2. Process Deviation Minus: This alarm type will activate if the process variable go below the current setpoint plus the current Deviation Minus setting.
3. Process Lo: This alarm should be set as an initial warning to the Operator that the process level is approaching the critical low level, LoLo.
4. Process LoLo: This alarm should be set as the critical low setpoint.
5. Process Hi: Process is approaching the critical Hi level.
6. Process HiHi: Critical high process level.

1.5 SYSTEM START UP

1.5.1 Temperature Warm-Up

1. The first, most critical step in system start-up is to ensure the entire system has reached a consistent operating temperature, usually 37° C.

1.6 OPERATIONS

1.6.1 Bringing items into the System:

1. Open Entrance Buffer Chamber (chamber closest to face of unit) door, disinfect chamber with 70% IPA and close.
2. Debag sterile items and immediately place inside Entrance Chamber. Repeat process until all items are transferred. Take care not to stack items to ensure that all surfaces are exposed during Oxygen flush.
3. Flush buffer chamber by dropping Oxygen concentration from 21% to 8%.

1.6.2 Bringing items out of the System:

1. When bringing items out of the system, containers should be closed and waste bags should be sealed.

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2. Pass items through pre sanatized Exit Buffer Chamber
3. Flush buffer chamber by dropping Oxygen concentration from 21% to 8%.
4. Remove items and put in appropriate passthrough.
5. Sterilize Exit Buffer Chamber and seal.

1.6.3 Use the Instruction Manual to Calibrate Sensors – Information on how to use the manual to calibrate sensors begins on page 1-76.

1.6.4 Relative Humidity Limit Control

1. After system temperature has reached the required operating level, RH Limit Control for each Incubation Chamber should be set to a low RH level, generally 50%.
2. Water source can now be introduced into the Incubation Chamber.
3. With a source of humidity present, the RH Limit Control will suppress RH levels to no higher than the setpoint. This allows the operator to incrementally increase the RH.
4. RH Limit Control can now be changed to a higher level.
5. When the RH for each chamber is reached, set the RH Limit Control to the required process specific RH level.
6. Once system temperature has been established and RH Limit Control has been established, additional controls can be turned on.

1.7 DISINFECTION

- The Xivo Incubation System must be disinfected before and after cell culture procedures to maintain aseptic processing. Ensure unit has been running for at least 30 minutes before disinfecting.
- Dampen a wipe with appropriate disinfectant.

- Place materials to enter the Xvivo System within the front 6 inches of the Laminar Flow Hood.
- Wipe all exterior surfaces of the materials with appropriate disinfectant and remove extra wrappings.
- Move items to an area pass the front 6 inches to within 3 inches of the back of the back of the Laminar Flow Hood.
- Allow time for the evaporation of the disinfectant, and place materials in the Buffer Module.

1.7.1 After Use Disinfection

1. Wipe all doorknobs with disinfectants
2. Wipe the floor of the Laminar Flow Hood from side to side, progressively moving from back to front. Be certain wiping path overlaps the previous path until the entire surface is wiped.

1.8 MAINTENANCE

1. The primary maintenance concern is replacement frequency of filters, the pre-Screen and the HEPA filter. Over time, as these filters become filled with particles, the effectiveness of the filters will degrade and air velocity of the Laminar Flow will decrease.
2. The Mini-Helic Pressure Gauge on the front of the Laminar Flow Hood measures back pressure upstream of the HEPA filter, in the plenum between the HEPA filter and the Pre-screen. This gauge will help determine when the filters need replaced.
3. Daily pressure readings should be conducted to help determine the required filter change frequency. Filters can be changed out annually for HEPA, with a monthly visual of Pre-screen. The filters can also rely on the Mini-Helic Gauge to determine when the change-out should occur.

1.8.2 Incubation Chamber

1. Microbial Filters should be changed after any adverse event or if there is any indication that they have become plugged or wet.
2. High flow filters should be changed after any adverse event or if there is any indication that they have become plugged or wet.

2.0 ATTACHMENTS

N/A

E. HISTORY

Effective Date	Revision	Change
07 Apr 2015	0	Original document
