STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Sterility Culture Using the BacT-Alert Microbiology System

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STCL-EQUIP-011
STERILITY CULTURE USING BACT/ALERT MICROBIOLOGY SYSTEM

1 PURPOSE

1.1 Products for cellular therapy and hematopoietic stem and progenitor cell transplantation should be sterile and free of contamination when administered to the recipient. The BacT/ALERT Microbial Detection System utilizes disposable culture bottles containing a liquid emulsion sensor that is monitored continuously using solid-state photodetectors to determine the amount of carbon dioxide (CO2) that is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium. When CO2 is produced, the color of the sensor changes from green to yellow.

2 INTRODUCTION

2.1 The LED (light emitting diode) projects light onto the sensor; the light reflected is measured by the photodetector. As more CO2 is generated, more light is reflected. This information is transmitted to a computer where it is compared to the initial CO2 level in the bottle. If there has been a sustained acceleration in the rate of CO2 production, high initial CO2 content, and/or an unusually high rate of CO2 production, the sample is determined to be POSITIVE. If after a specified number of days at these optimal conditions, the CO2 level does not change significantly, the sample is determined to be NEGATIVE.

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Director, Laboratory Manager, designated Stem Cell Laboratory personnel, and Quality Systems Unit are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	CO2	Carbon dioxide
4.2	ISBT	International Society Blood Transfusion
4.3	CBU	Cord Blood Unit
4.4	STCL	Stem Cell Laboratory
4.5	EPIC	Duke's Hospital Information System
4.6	QA	Quality Assurance
4.7	LN2	Liquid Nitrogen
4.8	mL	milliliter
4.9	CAPA	Corrective Action Preventative Action
4.10	PBPCs	peripheral blood progenitor cells

4.11 BM bone marrow

4.12 UCB umbilical cord blood

4.13 OOS Out of Specifications

5 MATERIALS

5.1 Supplies

5.1.1 Aerobic Culture Bottles

5.1.2 Anaerobic Culture Bottles

5.1.3 ChloraPrep SEPP® applicators

5.1.4 Alcohol prep pads (alternate)

5.1.5 Syringes

5.1.6 Needles

6 EQUIPMENT

6.1 BacT/ALERT 3D Microbial Detection System

6.2 Computer/Keyboard/Interface Box/Barcode Reader

7 SAFETY

7.1 Wear all appropriate personal protective equipment when handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, etc.

7.2 Discard culture bottles in the biohazardous trash after bottle data has been verified upon unloading.

8 PROCEDURE

8.1 Label each set of culture bottles with all pertinent information to include, but not limited to, ISBT 128 barcode labels, recipient's name, recipient's history number, donor's name (if applicable), donor's history # (*if applicable*), date, time, and the initials of technologist who inoculated the bottles.

8.2 Obtain a specimen for sterility testing using standard aseptic technique. A minimum of one mL of umbilical cord blood, bone marrow, peripheral blood progenitor cells, etc. should be inoculated into each of the two sterility culture bottles. Supernatant expressed off during processing of any or all of these cellular products can be used for inoculation.

NOTE: There may be specific instructions in other processing procedures, regarding inoculation volume, etc so be sure to follow those specific guidelines.

8.3 While working inside the biological safety cabinet using aseptic technique, remove the vent caps from both the aerobic and anaerobic culture bottles.

8.4 Clean the rubber septum (top) of each bottle thoroughly using a ChloraPrep® SEPP applicators (*one for each bottle*).

NOTE: Thoroughly swab bottle septums using ChloraPrep® SEPP applicators and allow it to sit for at least 30 seconds (*per supply instructions*) BEFORE inoculating the sample into the bottles.

8.5 **NOTE:** Alcohol prep pads can be used to clean the tops of the culture bottles if/when ChloraPrep® SEPP applicators are not available due to back orders, etc.

8.6 Using aseptic technique, inoculate each of the culture bottles with the specimen.

8.7 Complete the *BacT/Alert Log* and remove and affix the bottle barcode from the aerobic and anaerobic bottles onto the log sheet beside the appropriate product entry. Each bottle barcode will provide a link to the culture bottles used to inoculate the donor/recipient's cellular product specimen.

8.8 Log into the Observa software on the computer by entering a user name and password.

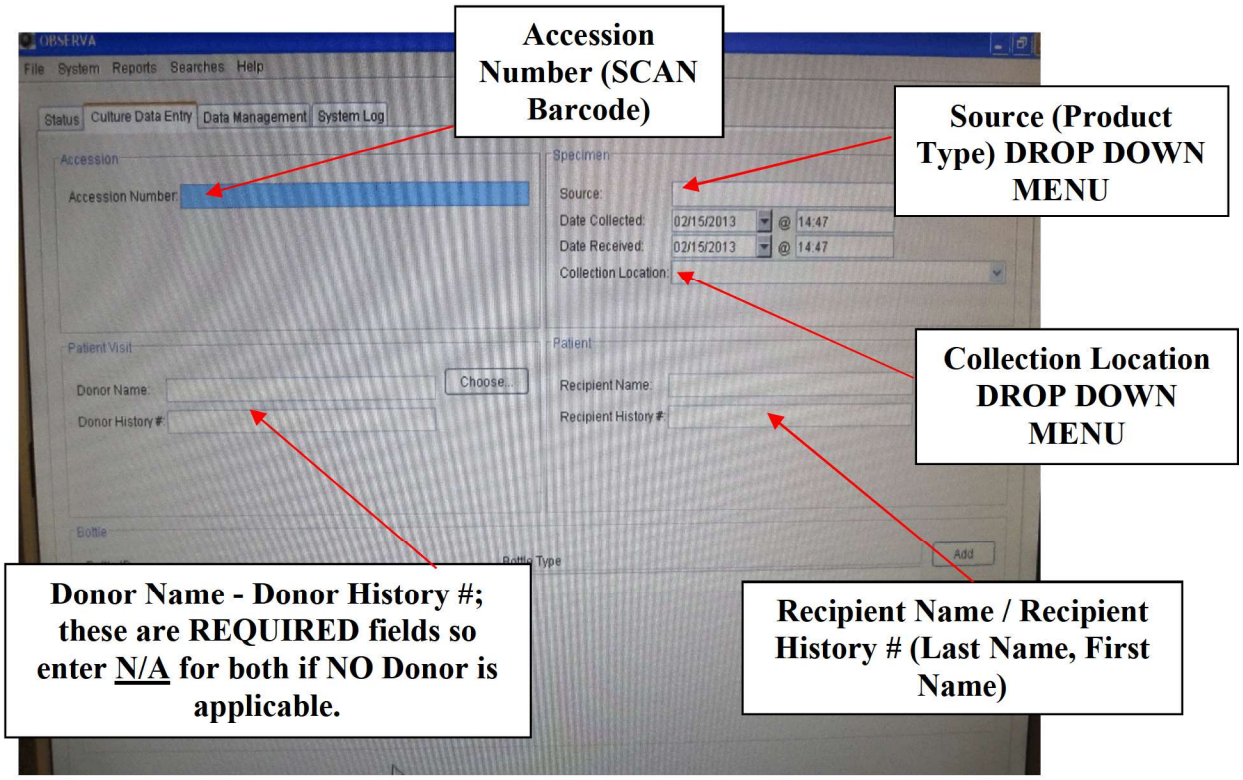
8.9 Select Culture Data Entry tab.

8.10 Scan the product ISBT barcode into the Accession field.

8.11 Select the appropriate product type from the drop down menu in the Source field.

8.12 Select the appropriate collection location from the drop down menu in the Collection Location field.

8.13 Enter the appropriate donor and recipient information as applicable.



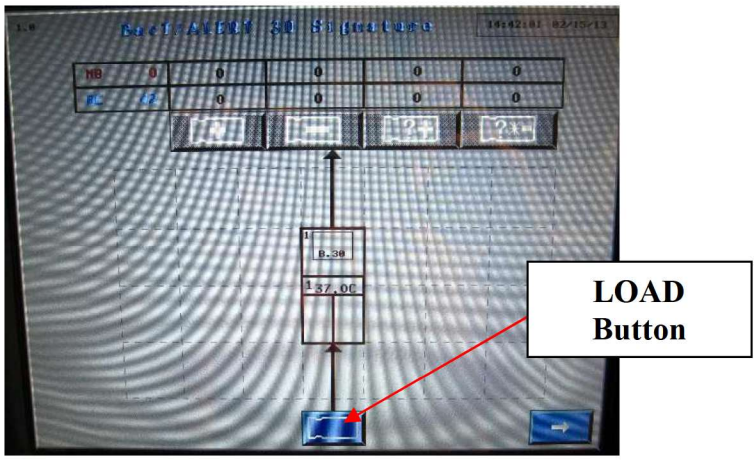
8.14 Scan the barcode from each culture bottle into Bottle ID field.

8.15 Click on SAVE.

8.16 Return to the STATUS screen and Log Off after completion of the entries.

8.17 Once all of the information has been entered into the Observa computer, the bottles are ready to be loaded into the BacT/ALERT 3D system.

8.18 To load bottles, press the LOAD BOTTLES button.



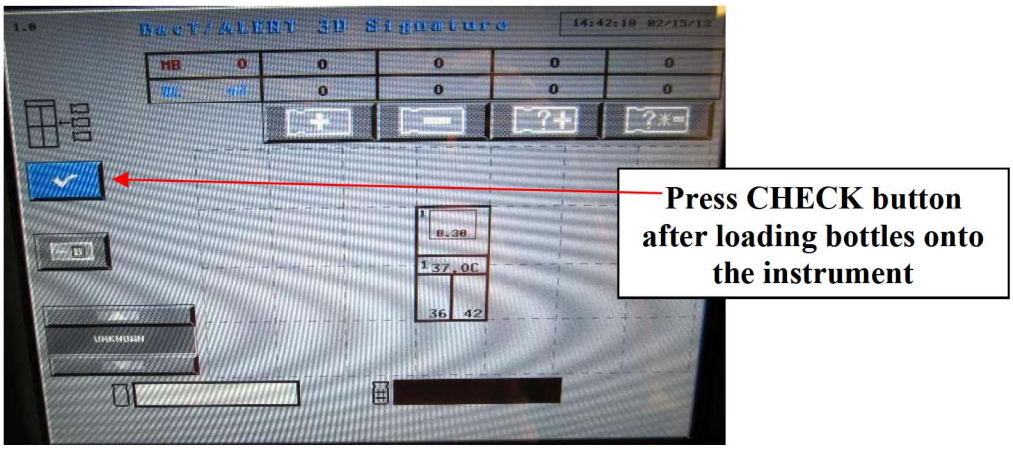
8.19 The Load Bottles screen will display. After scanning the Bottle ID, the Accession Number should be displayed at the bottom of the screen. If the Accession Number field is blank, do NOT load the bottles but repeat previous step to ensure all information is captured in the BacTAlert.

NOTE: Bottle ID and Accession Number are both required fields.

8.20 Drawers with available cells will have an illuminated green light.

8.21 Place the bottle in any available cell that has an illuminated green light.

8.22 Repeat steps 8.18 - 8.20 until all the inoculated bottles have been loaded into the BacT/ALERT 3D system. In order to maintain a stable internal temperature, it is recommended that the drawers NOT be left open for longer than 3 minutes. If it will take longer than 3 minutes to load the bottles accumulated, close the drawer and wait for the internal temperature to equilibrate before loading the remainder of the bottles.



8.23 Ensure drawers are completely closed. Press the Check button.

8.24 The culture bottles will remain on the instrument for a minimum of 7 days (unless instructed otherwise based on protocol requirements, etc).

8.25 Sub-Culturing Positive Cultures and Organism Identification

8.25.1 Clinical specimens (*including PBPCs, BM, UCB, and any other clinical specimens being handled / inoculated in the Stem Cell Laboratory*) identified as having a "positive culture" will be forwarded to the Microbiology Laboratory so the organism can be identified.

8.25.2 **NOTE**: For all clinical products, e-mail the medical team and text page the attending physician (*when appropriate*) immediately upon identification of a positive culture bottle(s) in the STCL.

8.25.3 Remove the bottles from the BacT/ALERT system using *STCL-EQUIP-011 (JA1) Unloading the BacT/ALERT Microbiology System and Printing Reports*. Place the pre-inoculated positive culture bottle(s) in a zip lock bag accompanied by an EPIC label reflecting the order for a stat gram stain and culture/sensitivity. Include instructions for the Micro Lab to call or page designated laboratory staff so results of the gram stain can be reported to the clinical team as soon as those results are available. Final results, as information is available, will be updated in EPIC so the clinical team has access to the information and can best treat the recipient and/or donor.

8.25.4 The "stat" gram stain result should also be reported to the attending physician by e-mail and text page (*if requested*) immediately upon notification of the results. The antibiotic coverage for the recipient can be evaluated by the clinical team to ensure coverage is adequate, based on the findings of the gram stain.

8.25.5 If the positive culture is obtained from a clinical product that has been collected before a recipient's transplant, the physician may opt to collect additional cells from that recipient/donor before taking the recipient to transplant.

8.25.6 Document the date, time, and person (*physician/clinical team staff*) who was contacted regarding the positive culture results. This information should be filed in the laboratory records and updated in the STCL's EMMES Database system.

8.25.7 A notation should be recorded on the laboratory file reflecting that the cellular product tested positive when cultured.

8.25.8 An OOS form (*STCL-EQUIP-011 FRM2 OOS – Product Sterility*) should be initiated to investigate a true positive culture (*see note*). A CAPA and/or an investigation may also be required given the situation and the investigation and/or follow up needed.

NOTE: If positive culture bottles from the STCL that are forwarded to the Microbiology Laboratory reveal "No Organisms Seen" and the final culture is reported as "Negative" or "No Growth", it is considered a "false positive" and a deviation would not be submitted.

8.25.9 If an attending physician has determined that a cellular product, with a positive culture, should be infused to the recipient, initiate a *Non-Conforming Product* form (STCL-QA-007 FRM1) and obtain the appropriate signatures authorizing the infusion of the non-conforming product.

8.25.10 If a cellular product with a positive culture has been cryopreserved, make sure that the product is stored in a LN2 vapor freezer since vapor storage is considered a "**virtual quarantine**". If the contaminated product is going to be used in the future and is currently stored in the liquid phase of LN2, it will need to be relocated to a designated vapor LN2 freezer to minimize cross-contamination risks.

8.25.11 If a cellular product with a positive culture has been identified by the attending physician for discard, initiate a *Record of Discard* (*STCL-SOP-045 FRM1*) and follow the steps as outlined in *STCL-QA-007 Non-Conforming Products – Receipt, Processing, Distribution, and Disposition*.

8.25.12 Sterility results are gathered and reported at the joint QA Committee meeting on a quarterly basis.

8.25.13 Results are monitored within the STCL on a monthly basis to detect trends and to isolate specific organisms that might identify a system

error in collection or processing. The STCL's positivity rate should be ≤5%.

8.25.14 **NOTE**: Quality control specimens, using known + and – controls, are tested in the STCL on a monthly basis. QC results will be recorded, signed, and dated on *STCL-FORM-076 BacT-Alert Monthly Quality Control Log* to meet regulatory requirements.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-EQUIP-011 FRM1 BacT-Alert Log Sheet

9.2 STCL-EQUIP-011 (JA1) Unloading the BacT/ALERT Microbiology System and Printing Reports

9.3 STCL-QA-007 Non-Conforming Products – Receipt, Processing, Distribution, and Disposition

9.4 STCL-QA-007 FRM1 Non-Conforming Products

9.5 STCL-SOP-045 FRM1 Record of Discard

9.6 COMM-QA-042 Deviations and Investigations

9.7 STCL-EQUIP-011 FRM2 OOS – Product Sterility FRM2

9.8 STCL-FORM-076 BacT-Alert Monthly Quality Control Log

10 REFERENCES

10.1 BacT/ALERT User Manual

10.2 American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Product. Current edition.

10.3 Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT) and Netcord. International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release Current edition.

10.4 Comparison of automated culture systems with a CFR/USP-compliant method for sterility testing of cell-therapy products.
HM Khuu, F Stock, M McGann, CS Carter, JW Atkins, PR Murray, and EJ Read
Cytotherapy, Jan 2004; 6: 183-95

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
15	Barbara Waters-Pick	• Added Section 8.25.14 to discuss testing of + and – QC controls • Added STCL-FORM-076 BacT-Alert Monthly Quality Control Log to Section 9.8

Signature Manifest

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