

The Necessity of Equipment Performance Qualification In A Laboratory Setting

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Abstract

Determining potency of manufactured cord blood products is required before release for transplantation. One method of detecting functionally viable cells is the Colony Forming Unit assay (CFU). To create the environment suitable for growth, the St. Louis Cord Blood Bank (SLCBB) uses water-jacketed incubators to provide stable temperature, high humidity and controlled CO₂ for incubation of CFU culture plates. In October of 2007, the performance of the bottom incubator of this equipment set became suspect due to observation of questionable colony growth in CFU plates. Use of this instrument was suspended pending re-qualification.

Operational Qualification (OQ) was performed by evaluation of documented operational parameters: temperature, water level and CO₂ level. Since all parameters were acceptable and constant over time, Performance Qualification (PQ) was initiated. The procedure for the performance qualification study began with duplicate incubation of CFU plates in the top and bottom incubators and the results for this initial sample indicated a significant variance in total colony growth (p<0.01). Seven plates from the bottom incubator showed no growth.

Initial investigation revealed that all recorded parameters (manual temperature, digital CO₂%, visual water level) remained acceptable. Further investigation included: 1. Analysis of data provided by the Rees continuous monitoring system 2. Consultation with the manufacturer of the CFU media, and 3. Onsite inspection of the incubator by a contracted vendor.

Rees data analysis showed that when the chamber was depleted of CO₂, there was a short term overcompensation of CO₂, twice that designated for the environment. Though not sufficient in duration to trigger an alarm, the conditions adversely impacted CFU growth. The investigation of the incubator revealed that a piece of tubing connected to the bottom CO₂ sensor had deteriorated and cracked. When the internal environment was depleted of CO₂, external air entered the damaged tubing, resulting in the relay of incorrect information to the sensor. The false reading by the sensor caused the injector to overshoot the amount of CO₂ needed to replenish the chamber percentage. The tubing was replaced and the sensor and injector were both recalibrated.

The SLCBB instituted a follow-up performance qualification mirroring the original study design. The results of this study demonstrated an acceptable variance between the top and bottom incubator (p=0.76), and the incubator was deemed suitable for use.

The St. Louis Cord Blood Bank's policy to perform Installation Qualification, Operational Qualification, and Performance Qualification protected the bank from unnecessary loss of data integral to the qualification and release of cord blood products. Installation Qualification and Operational Qualification alone would not have been sufficient to diagnose the equipment failure.

Background

The St. Louis Cord Blood Bank (SLCBB) at SSM Cardinal Glennon Children's Medical Center facilitates the collection of cord blood donations from 29 hospitals in Missouri and Illinois. Collections are manufactured by red cell reduction and plasma depletion, and cryopreserved as a source of cells for transplantation and other cellular therapies. Since its establishment in 1995, 80,000 mothers have donated their cord blood to the SLCBB, creating an inventory of nearly 20,000 products from which 1,500 products have been distributed.

The potency of each cord blood unit at the SLCBB is determined by the evaluation of the following criteria: Total Nucleated Cell count (TNC), CD34+ enumeration, Trypan Blue viability, and Colony Forming Unit assay (CFU). The CFU assay is widely accepted as an indicator of product proliferative capacity. Cord blood products meeting acceptability criteria, including CFU growth which exceeds established thresholds, are authorized for release by the SLCBB.

Optimal incubation environment for the CFU assay is achieved using water-jacketed incubators to provide stable temperature, high humidity and controlled CO₂. SLCBB incubators are continuously monitored for temperature and CO₂ fluctuations using a computerized environmental surveillance system.

In October of 2007, the performance of the bottom incubator of in this equipment set became suspect due to observation of questionable colony growth in CFU plates. Use of this instrument was suspended pending re-qualification.

Materials & Methods



1. Location of tubing deterioration
2. CO₂ sensor

Materials & Methods (continued)

The re-qualification study is detailed below:

OPERATIONAL QUALIFICATION (OQ)

In order to operationally qualify this piece of equipment, critical parameters were monitored for a period of three months. These parameters included:

- Temperature
- CO₂ levels
- Water level

PERFORMANCE QUALIFICATION (PQ)

Specimen:

CFU samples from HPC, Cord Blood post processing, dilution factor (x 16), volumes calculated to inoculate 1 x 10⁶ cells per 3mL MethoCult media.

Procedure:

- Duplicate CFU assays will be simultaneously inoculated and plated by the processing technologist according to standard operating procedure.
- One set of plates will be placed in the bottom incubator in room 148 and the other set of plates will be placed in the top incubator in room 148. A total of 10 tests will be incubated in the bottom incubator. The top incubator currently in use in Room 148 will be used as a standard.
- After an incubation period of fourteen days:
 - plate media will be evaluated for lack of bacterial or fungal growth, demonstrating the ability of the incubator to maintain sterile conditions;
 - the CFU assays will be scored and compared between the incubators. All scoring related to this study will be performed by the same technologist. If the results from the incubators correlate within 85% of the standard, the second phase of testing will proceed.
- Concurrent testing will consist of a further 15 singlet samples to be incubated in each top and bottom incubators. After the fourteen day incubation period the assays will be scored. Results will be compared to a reference range based on historical data.

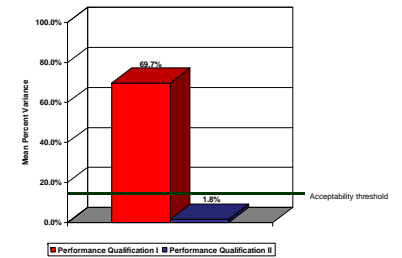
Procedure Notes:

An historical range based on CFU's scored for the year of 2007 is used to establish critical limits. The year 2007 is significant as it represents scores reported by all technical staff that are currently responsible for reporting CFU assay results. Historical data will be expressed as a CFU/TNC ratio, as it corrects for TNC and, therefore, provides a more consistent measure of performance than absolute CFU count. The SLCBB has determined that the CFU/TNC ratio is a cell dose independent measure of a cord blood graft's hematopoietic potential and is strongly correlated with speed of engraftment.*

* The Ratio of Colony Forming Unit (CFU) to Total Nucleated Cell (TNC) Count Predicts Engraftment in Umbilical Cord Blood Transplants. B Triplett MD, D Regan, J Wofford, J Schaeffler, W Ferguson MD, St Louis Cord Blood Bank, Abstract poster presentation, ASBMT 2008.

Results

Prospective Results: Absolute Colony Count Variance Top Incubator vs. Bottom Incubator



- Prospective results from PQ I were unacceptable, necessitating further investigation
- PQ II was initiated after repair to CO₂ sensor tubing and yielded prospective results within SLCBB acceptability limit (variance <= 15%)

	PQ II Concurrent Results	Historical Data
N	15	1,773
Minimum	6.6	0.6
Maximum	16.2	38.6
Mean	13.4	10.4
Median	13.7	9.9
SD	2.6	3.8
Range ± 2SD	8.1 – 18.6	2.8 – 18.0

Discussion

- The SLCBB adopts its quality control template from AABB Quality System Essentials (QSEs).
- The St. Louis Cord Blood Bank's policy to perform Installation Qualification, Operational Qualification, and Performance Qualification protected the bank from unnecessary loss of data integral to the qualification and release of cord blood units.
- Installation Qualification and Operational Qualification alone would not have been sufficient to diagnose the equipment failure and we therefore recommend that IQ, OQ, and PQ be central components of any laboratory quality plan.