Verification and Validation of ATP Bioluminescence Assays for Hematopoietic Stem Cell Therapy

How does the CFU Assay Compare?

Reasons Why ATP Bioluminescence Assays Correlate with the CFU Assay

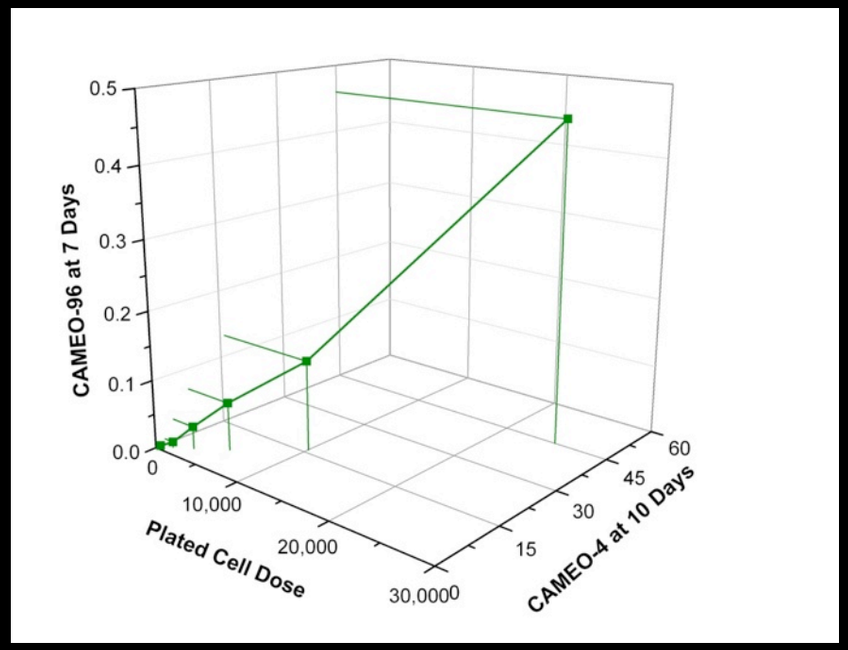
Assay Verification

- HALO® was originally derived from the "classic" methylcellulose CFU assay using the same reagents, but a more advanced, instrument-based, ATP bioluminescence readout that is non-subjective, standardized and validated. The original HemoGenix® methylcellulose ATP bioluminescence assay is now called CAMEO™-96.

- HALO® is methylcellulose free and measures the proliferation process required for division of the cells that will eventually produce a colony of differentiated cells that can be identified and counted. HALO® can therefore predict the outcome of a CFU assay in less than half the time it takes to grow colonies and allow the cells to differentiate and mature.

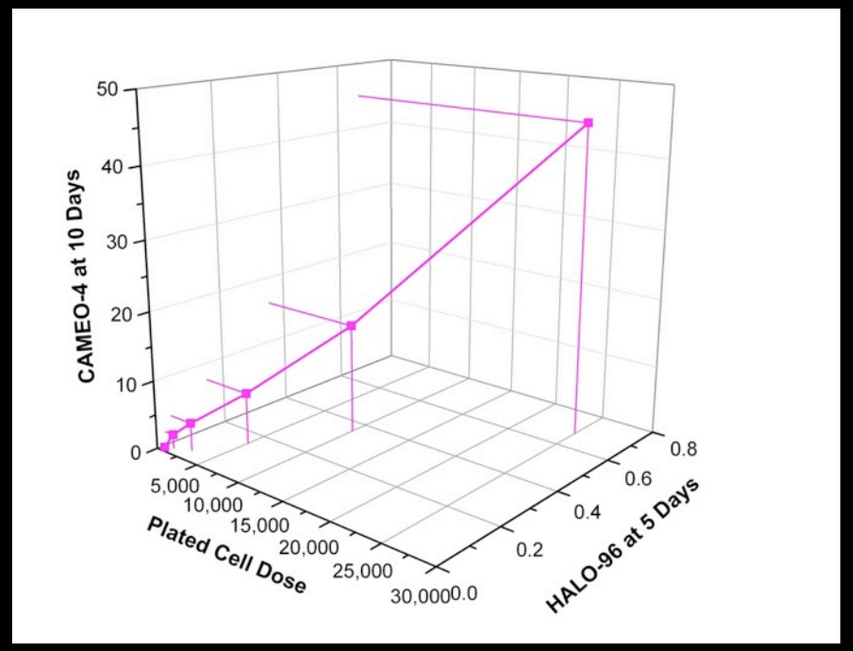
- If the total number of colonies counted is plotted against the ATP concentration, a straight line, linear regression should be produced. The diagrams shown in the next slide demonstrate that when total colony number is plotted against ATP concentration as a function of cell dose, a high correlation coefficient (R) is always obtained.

Verification of the CFU Assay with ATP Bioluminescence Assays



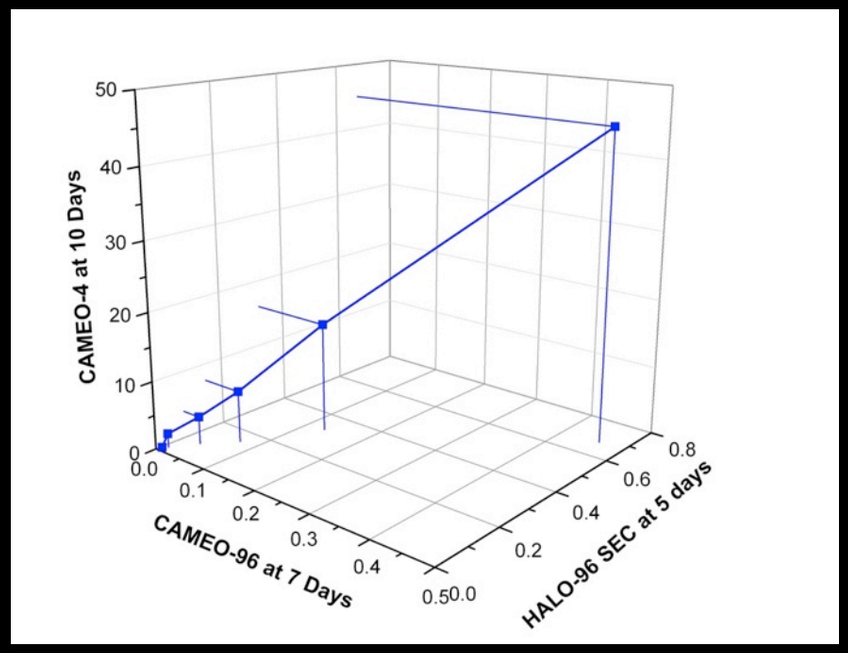
Cells vs CAMEO-4	R = 0.99
Cells vs CAMEO-96	R = 0.993
CAMEO-4 vs CAMEO-96	R = 0.997

Cell Dose:
500 cells/well
1,000 cells/well
2,500 cells/well
5,000 cells/well
10,000 cells/well
25,000 cells/well

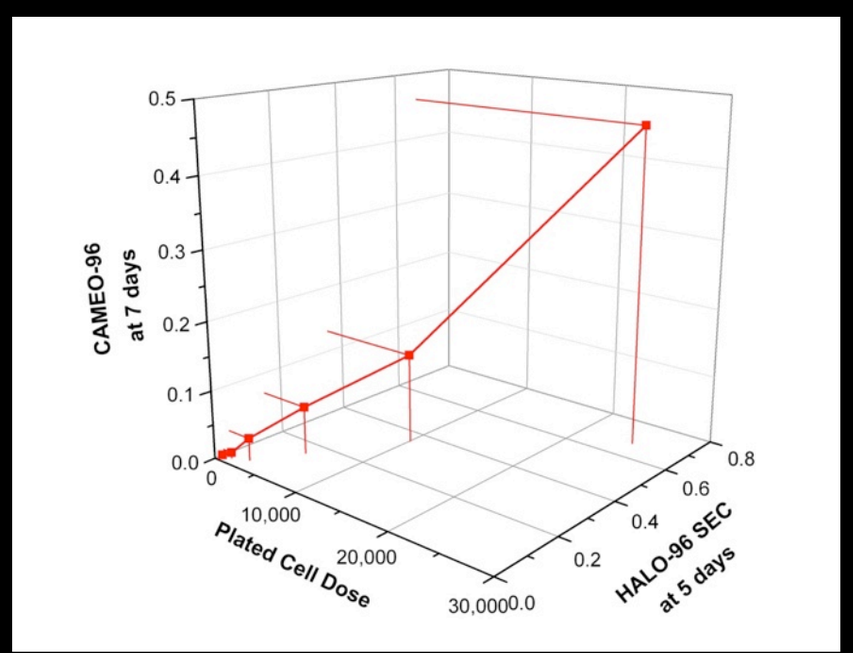


Cells vs CAMEO-4 (CFU)	R = 0.99
Cells vs HALO	R = 0.987
CAMEO-4 vs HALO	R = 0.986

Has the CFU assay been verified against any other assay?



CAMEO-4 (CFU) vs CAMEO-96	R = 0.995
CAMEO-4 (CFU) vs HALO	R = 0.986
CAMEO-96 vs HALO	R = 0.964



Cells vs CAMEO-96	R - 0.993
Cells vs HALO	R = 0.987
CAMEO-96 vs HALO	R = 0.964

Standardizing the CFU Assay

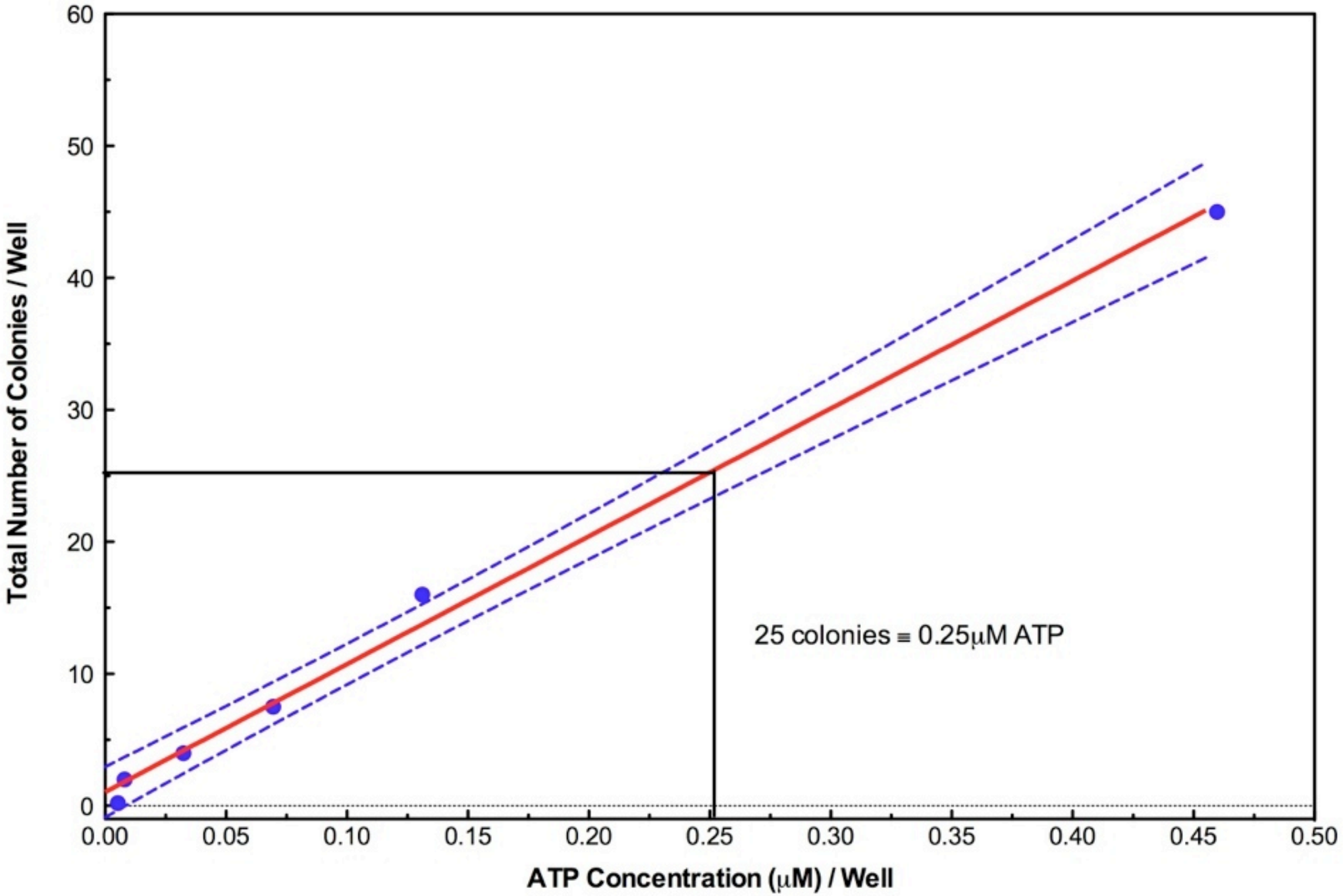
Is the Commercial CFU Assay Actually Standardized?

- Contrary to reports (using reagents or even automated counting methods), the CFU assay is not standardized because there are no external standards and controls to calibrate and standardize the assay.

- Only ATP bioluminescence assays can back-standardize the CFU assay.

- Only ATP measurements quantify cell growth capability to provide meaningful results.

Standardizing the CFU Assay using HALO®



ATP Bioluminescence Assay Properties

Can the CFU assay produce similar properties?

- ATP assay correlation coefficient (R) => 0.997
- ATP Standard Curve Slope = 0.937 (range: 0.8 -1.1)
- Assay ATP sensitivity: ≤ 0.001µM
- Assay cell sensitivity: 20-25 cells/well
- Low ATP control range: 0.043µM - 0.058µM
- High ATP control range: 0.595µM - 0.805µM
- Extra high ATP control range: 1.488µM - 2.013µM
- Lowest ATP value indicating unsustainable cell proliferation: =< 0.04µM
- ATP value below which cells are not metabolically viable: =< 0.01µM

ATP Bioluminescence Assay Validation Parameters

Does the CFU assay produce similar validation parameters?

- Cell-Based Assay ATP Linearity: ~4 logs
- Accuracy (% correct outcomes): ~95%
- Sensitivity and Specificity (Receiver Operator Characteristics Curve Fit): 0.73 - 0.752 (range: 0.5-1)
- Precision (Reliability & Reproducibility): =<15%
  (Lowest Level of Quantitation, LLOQ: =< 20%)
- Robustness (Intra- & inter-lab): => 95%
- Z-Factor (high throughput capability): >0.76 (range: 0.5-1)

Advantages of Using ATP Bioluminescence Assays for Hematopoietic Cell Therapy

- Shown to be an alternative to and replacement for any CFU assay.
- Accepted as alternative assays by the FDA, AABB and FACT.
- Cell culture for only 5-7 days and instrument-based results within 10 minutes.
- Includes external standards and controls.
- No need for costly proficiency testing. The assays are calibrated and standardized so that you know that the assay is working correctly and the results will be reliable.
- Allow results to be directly compared over time. Important for comparison of procedures and accumulation of historical data.
- Completely validated according to FDA regulatory requirements.
- Assays designed for specific applications. Do not have to reinvent the wheel to adapt the same assay for different purposes.
- Taking supplies, time and personnel costs into consideration, assays are far more cost-effective to perform.