



WHITE PAPER

A Cost-Analysis of Colony-Forming Assays and Equivalent Alternatives used to Determine Growth of Hematopoietic Cells Derived from Umbilical Cord Blood, Normal and Mobilized Peripheral Blood and Bone Marrow.

April, 2010

This White Paper is a forward-looking statement. It represents the present state of the art and future technology in the field of stem and progenitor cell testing. The views expressed in this White Paper are those of HemoGenix®, Inc.

Changing the Paradigm

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Introduction

The colony-forming unit (CFU) assay for human blood-forming stem and progenitor cells has been used in the clinical field since 1970 [1]. Indeed, the CFU or colony-forming cell (CFC) assay continues to be used in the clinical arena, particularly in umbilical cord blood (UCB) banking and processing. As a product that is usually cryopreserved and stored for long periods of time (10-15 years), knowing whether the cells of a UCB unit will grow is obviously of some importance. Interestingly, however, the assay is usually performed prior to cryopreservation, rather than after storage when quality, potency and release criteria of the thawed cells should be known prior to transplantation [2,3].

Nevertheless, the CFU/CFC or an equivalent assay is a requirement in the UCB processing laboratory [4,5,6] and often still used for mobilized peripheral blood (MPB). Like UCB, mobilized peripheral blood is also cryopreserved, but for much shorter time periods before it is used. In contrast to bone marrow, both UCB and MPB are now preferred products for transplantation purposes. Yet despite new and improved transplantation regimen, there has been little initiative or innovation to improve the tests and assays performed to characterize the manufactured products. Although considerable criticism can be aimed at the use of these tests and assays from a scientific viewpoint, the aim of this White Paper is to demonstrate that time and cost aspects of performing a particular procedure can be considerably reduced when new and improved tests and assays are implemented.

In fact, it is often thought that new technology is more complicated and expensive to perform. This may be one reason for continuing to use the same tests and assays, even though far superior assays are commercially available. For the CFU/CFC assay at least, this a misconception. A cost analysis of the CFU/CFC assays that have been used in the cord blood field since it started in 1989 [7], shows that they are actually more expensive to perform. Indeed, as we demonstrate below, new CFU/CFC equivalent assay platforms not only save time, but also money.

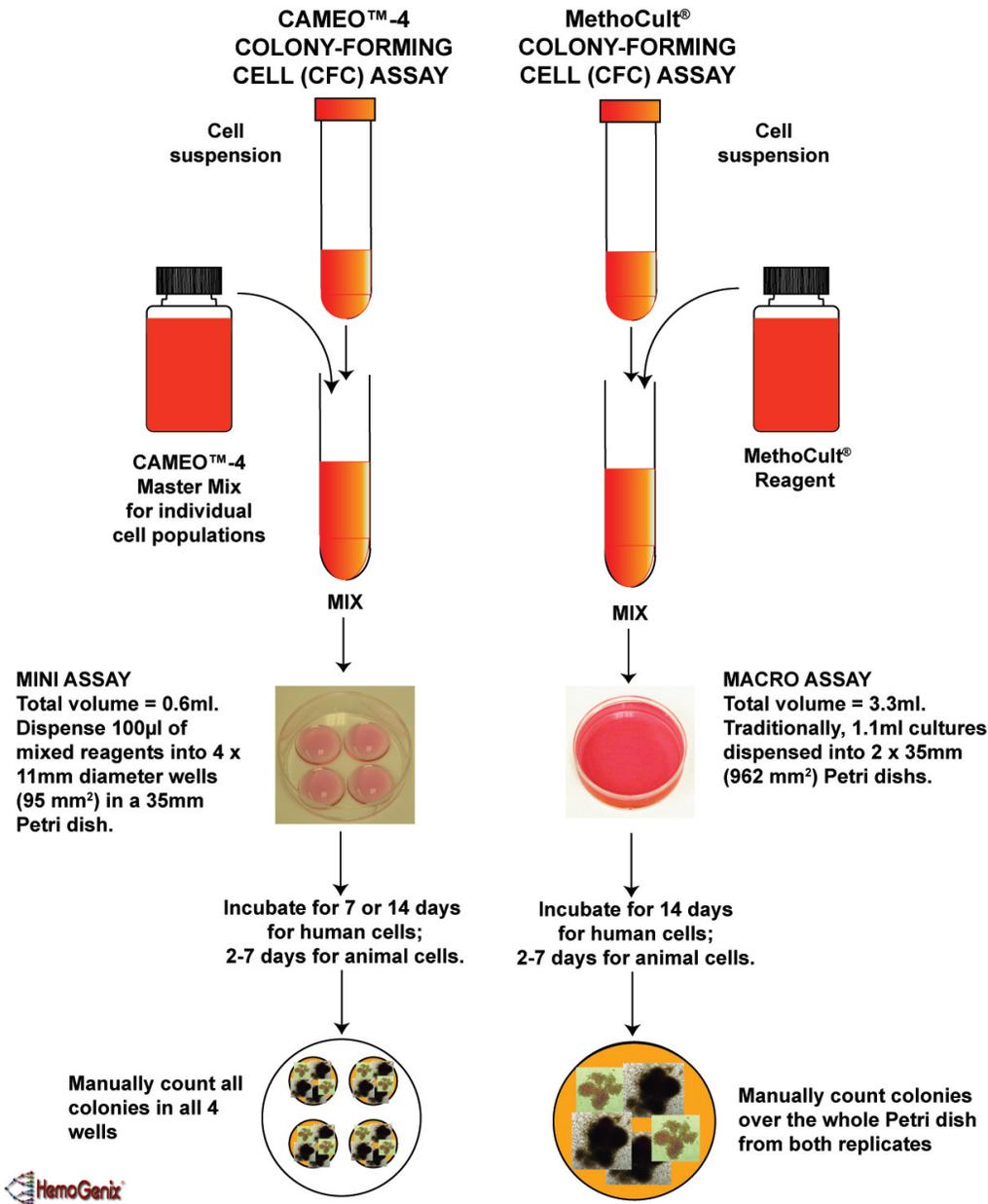
The Problem

When an assay is implemented, the cost of the assay does not stop when the reagents or assay kit is purchased. In most cases, instrumentation is required as well as extra supplies. Furthermore, the time to prepare extra reagents, labor costs and indirect costs also have to be taken into account. Once the assay has been completed, the results have to be tabulated, perhaps graphed, interpreted and documented. These too, take time and money and add to the overall cost of the assay. All of these aspects are part and parcel of performing a CFU/CFC assay, regardless of whether it is for one sample or multiple samples.

To demonstrate this, we will consider three different ways of performing the same CFU/CFC assay. The first two are manual assays and shown side by side in Fig. 1.

Figure 1. Comparison of Two Manual CFU/CFC Assays

CAMEO™-4 Compared to MethoCult®



The first assay is the traditional CFU/CFC assay and commercialized as MethoCult® from Stem Cell Technologies. Similar macro-type assays can also be obtained from Sigma-Aldrich and R & D Systems. In all cases, a reagent containing growth factors and cytokines mixed with other culture components is dispensed into a tube. This is followed by a specific volume of a target cell suspension adjusted to the correct cell concentration. The contents of the tube are mixed thoroughly. Using a syringe and needle, 2 x 1 (or 1.1) ml aliquots are dispensed into 35mm Petri dishes. For all cultures containing human cells, a 14 day incubation time is required. A more expensive version called MethoCult® “Express” is now offered and apparently can be performed in 7 days. After incubation, the plates are placed under an inverted microscope, usually equipped with at least 2 objectives, and the colonies manually counted.

The second assay, called CAMEO™-4 [8], and also shown in Fig. 1, is the equivalent of the

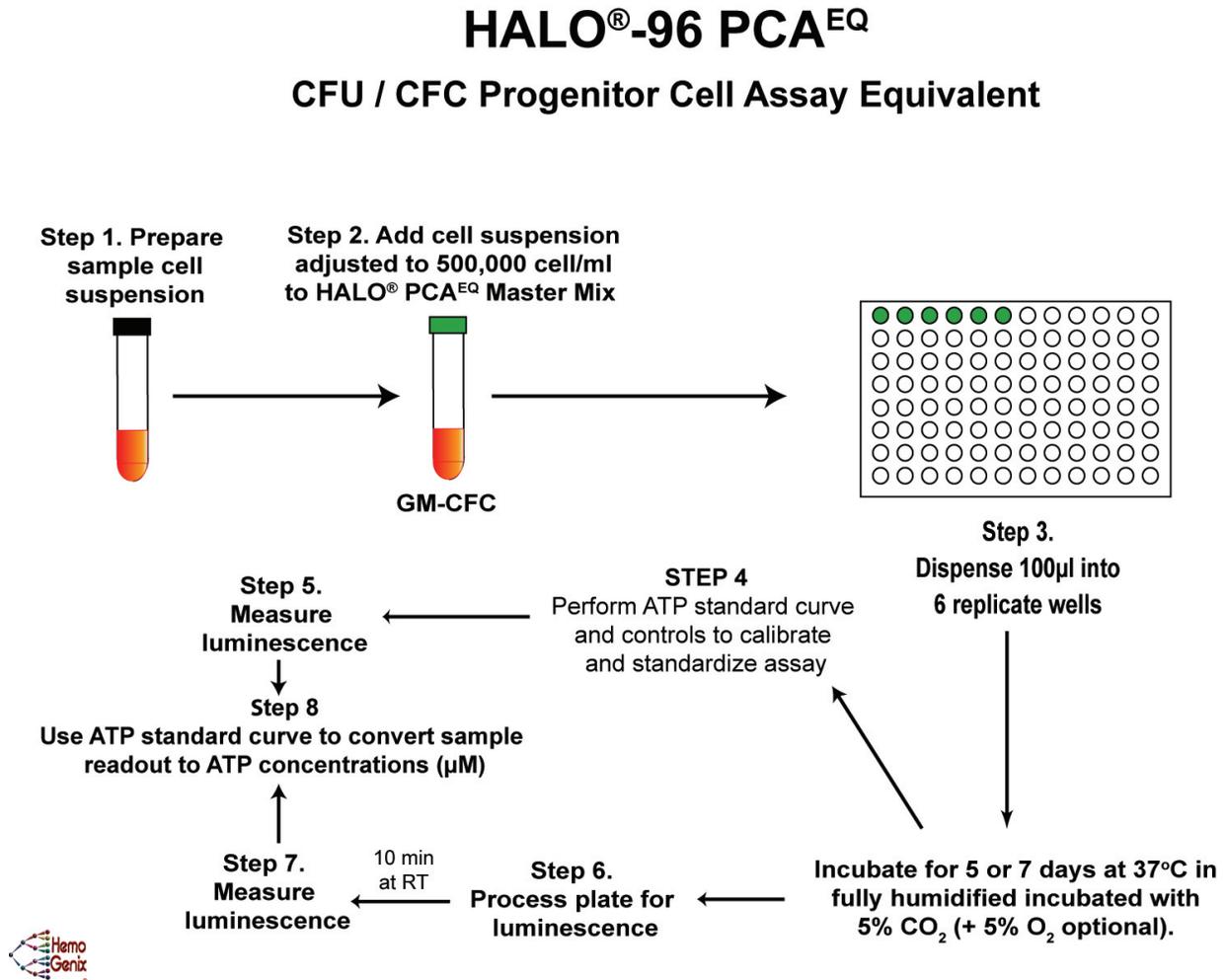
Changing the Paradigm

MethoCult® or MethoCult® “Express” assays, using the same growth factors/cytokines. CAMEO™-4 is a miniaturized CFU/CFC assay. Rather than using 35mm Petri dishes, it uses 35mm Petri dishes that contain 4 wells in which only 100µl of reagents are dispensed into each well. It follows that using CAMEO™-4 requires less reagent and fewer target cells. Human cells cultured using CAMEO™-4 can be incubated for 7 days (cf. MethoCult® “Express”) or 14 days (cf. MethoCult®). The reason why CAMEO™-4 can be completed in 7 days is because CAMEO™-4 uses the same reagents as the third CFU/CFC equivalent assay, namely HALO®-96, in particular, HALO®-96 PCA^{EQ} (Progenitor Cell Assay, Equivalent).

The HALO® Platform was first developed in 2002. At that time, it was developed as a methylcellulose assay. Unlike the traditional methylcellulose assays (e.g. MethoCult®), however, HALO® incorporated a non-subjective, ATP-based luciferin/luciferase bioluminescence readout that was completely instrument-based allowing results to be calculated and graphed automatically. Using intracellular ATP as the biochemical marker for proliferation of stem and progenitor cells allowed the assay to be calibrated and standardized against an ATP standard and controls. This, in turn, also allowed the assay to be validated [9]. During the development of HALO®, it was demonstrated that hematopoietic cells need not differentiate into colonies of mature cells. The proliferation of the cells at 7 days would predict the product and number of colonies at 10 and 14 days [8,9,10,11]. Thus, all methylcellulose HALO® assays using human cells are actually performed on day 7 of incubation. Since CAMEO™-4 uses the same reagents as HALO®, there is a direct correlation between the results of these two assays performed on days 7 or 14 [8,10]. The MethoCult® “Express” assay is therefore an attempt, (and as will be demonstrated below, an expensive attempt), to copy and provide a more rapid assay [10], despite the fact that the user still has to count cell aggregates and/or colonies. As seen later, this provides no cost-savings. In fact, it is the most expensive assay to perform.

HemoGenix® is not a company that stands still. At HemoGenix®, we are continually trying to improve our products. Such is the case with the HALO®-96 SEC assay system. The term SEC denotes “Suspension Expansion Culture”. This is a methylcellulose-free culture system that has several important advantages over methylcellulose. The latter is extremely difficult to dispense accurately and as a result, large coefficients of variation (%CV) are typical when methylcellulose is used. Since the SEC format does not require methylcellulose, normal pipettes and dispensing techniques can be used reducing the %CV to 15% or lower. It also allows faster assay setup. The lack of methylcellulose in the culture also allows cell interaction to occur. This not only has the effect of reducing the lag time for the onset of proliferation, but also increases the sensitivity by at least 2 fold. The result is an assay that can be completed in as little as 5 days, but is flexible enough to be performed on days 6 or 7. HALO®-96 PCA^{EQ} [12] incorporates SEC technology, but uses the same growth factors and cytokines as the MethoCult® assay reagents. The protocol for HALO®-96 PCA^{EQ} is shown in Fig. 2. Unlike CAMEO™-4 and MethoCult® however, HALO®-96 PCA^{EQ} is a 96-well plate assay in which 4 or 6 x 100µl of culture reagent is dispensed into each well. It is not necessary to use the whole plate at a time, since the assay kit includes sterile adhesive foils that keep unused wells sterile for later use. Like all HALO® assays, high and low controls are included to standardize the assay and an ATP standard allows calibration of the instrument, assurance that the reagents are working correctly and conversion of Relative Luminescence Units (RLU, instrument readout) to standardized ATP concentrations using the ATP standard curve. This, in turn, provides the ability to compare results within and between laboratories, an important aspect of the assay validation process. Indeed, this type of intra-laboratory results comparison using HALO® for UCB has been performed and published previously [13].

Figure 2
HALO-96 PCA^{EQ} Graphical Procedure

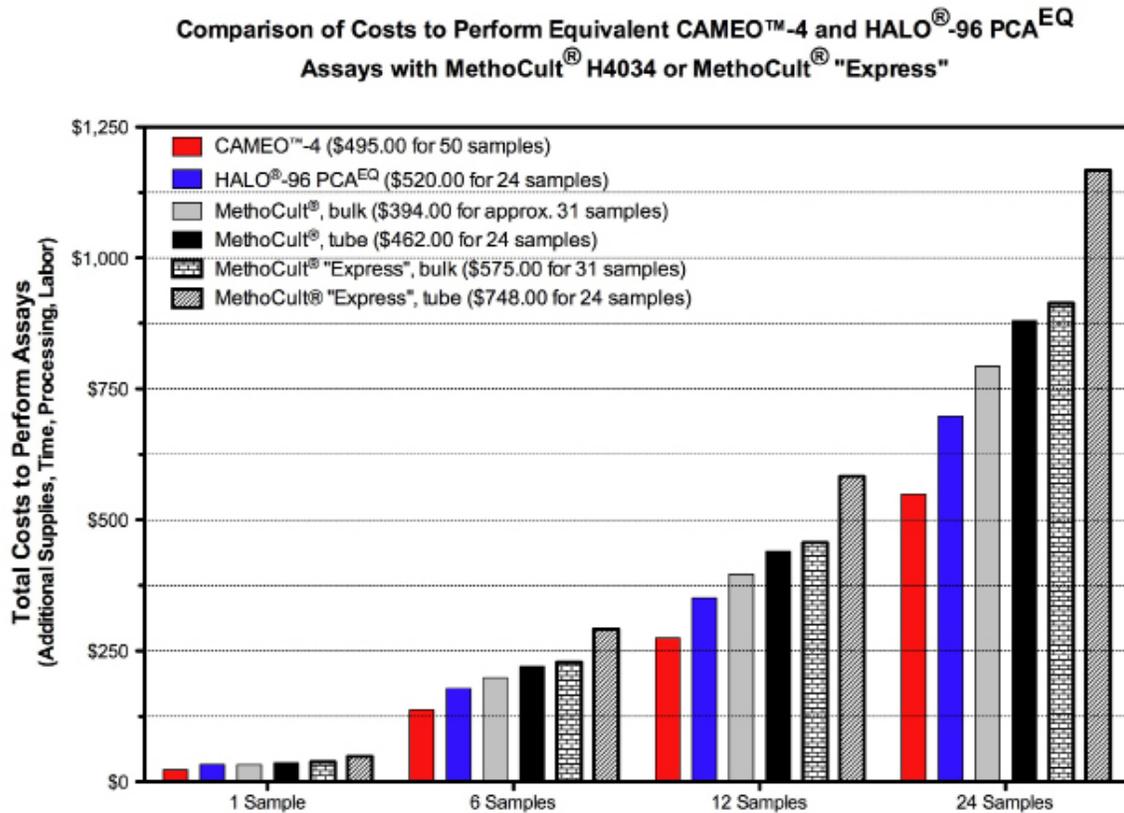


Which Assays are the most Cost-Effective to Perform? – The Solution

Tables 1 and 2 (at the end of this article) show the cost breakdown of performing CAMEO[™]-4 and HALO[®]-96 PCA^{EQ} compared to MethoCult[®] H4434, containing GM-CSF, SCF, IL-3 and EPO and commonly used in UCB and mobilized peripheral blood processing laboratories (Table 1), and MethoCult[®] “Express” (Table 2). These tables show the cost of the respective assays to purchase (excluding shipping and handling etc.), the individual cost/sample and the additional supplies required and their costs. The tables also show other characteristics of the assays as well as the approximate labor costs and the cumulative costs for different numbers of samples. From the calculated costs, it is clear that both CAMEO[™]-4 and HALO[®]-96 PCA^{EQ} appear to cost less to perform than either MethoCult[®] H4434 or MethoCult[®] “Express”, and this is born out by the dramatic savings incurred when either CAMEO[™]-4 or HALO[®]-96 PCA^{EQ} are used. In fact, it is possible to save between 7% to 20% using HALO[®]-96 PCA^{EQ} and more than 30% using CAMEO[™]-4 over MethoCult[®] H4434 or MethoCult[®] “Express” (Table 3). The actual costs to perform the different assays for 1, 6, 12 or 24 samples are shown diagrammatically in Fig. 3.

Changing the Paradigm

Figure 3



The reasons why CAMEO™-4 costs less to perform than its counterpart MethoCult® assay is because important supplies required for the assay are included with the CAMEO™-4 kit, especially the inclusion of 50 culture plates, allowing for 50 samples. Furthermore, since the assay incorporates a miniaturized format, lower reagent volumes are required. In addition, it takes less time to count colonies over a smaller growth surface.

The next question is, why does HALO®-96 PCA^{EQ} also cost less to perform than any of the MethoCult® assays? All HALO® assay kits include everything necessary to perform a quality, standardized and validated assay. Only the addition of cells is required. However, although most assays take a similar time to setup at the front end, all HALO® assays save time and money after the culture period has elapsed. This is because no manual counting is required. The assay is instrument-based and all results can be calculated, graphed automatically and documented using the software that controls the instrument. Figure 4 shows that when the number of samples increases, the time savings to perform the assay increases exponentially. This results in a dramatic savings in labor costs for as few as 6 samples (Fig. 5).

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Figure 4

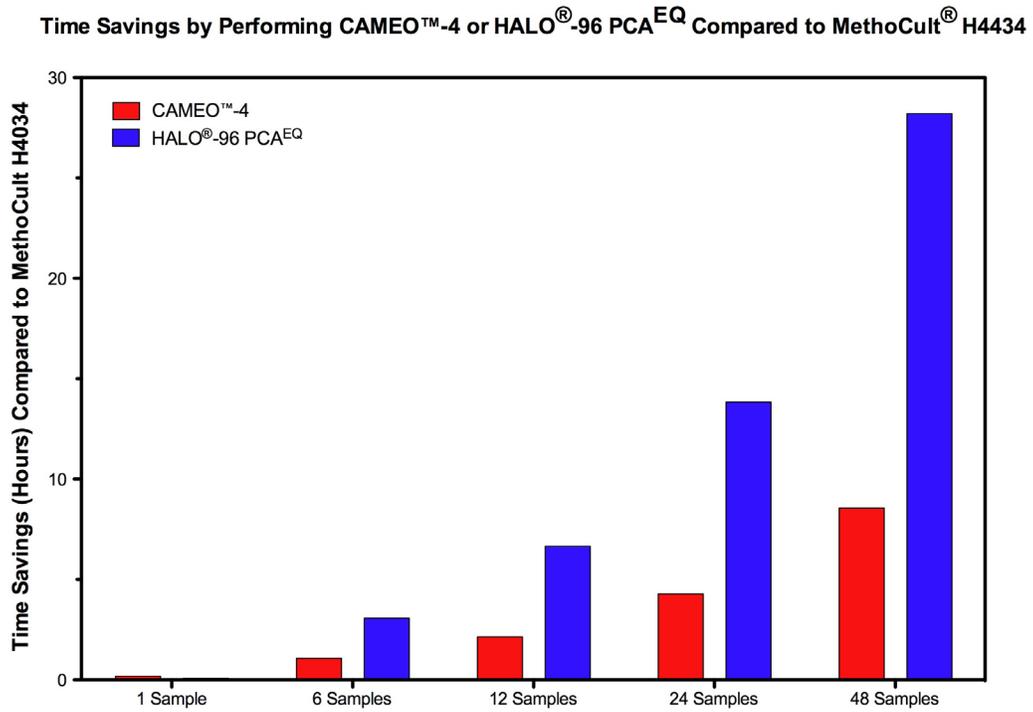
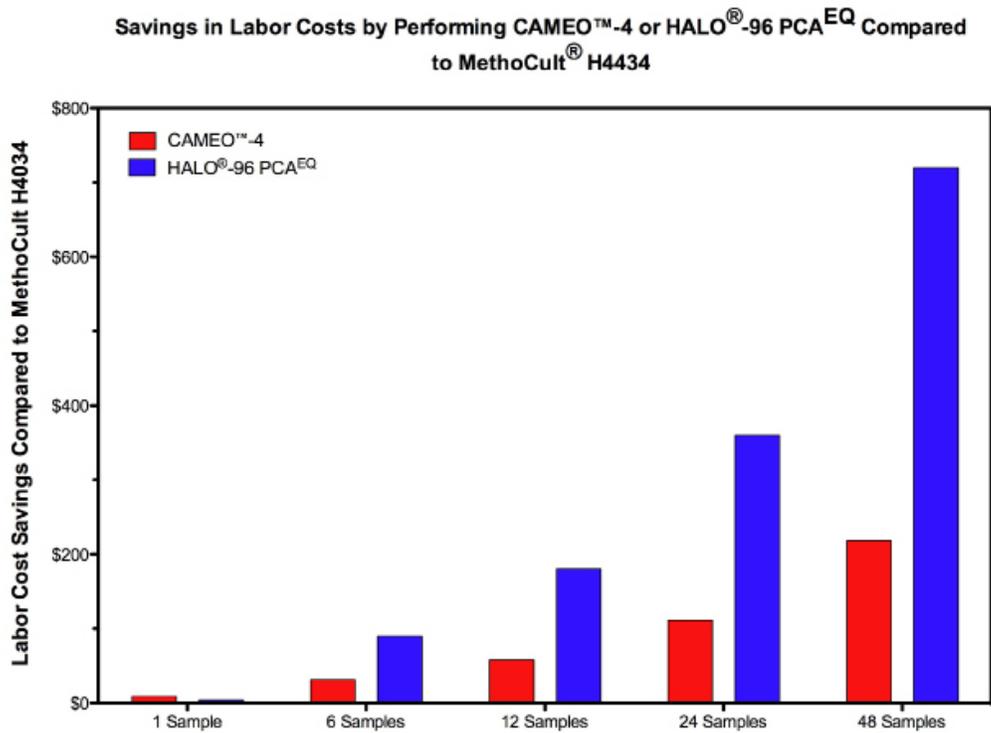


Figure 5



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Conclusions

The idea that new, advanced assays have to be more expensive is a false assumption. Although CAMEO™-4 is the most cost-effective CFU/CFC assay available, it suffers from the same problems as the MethoCult® assays in that they are subjective. Associated with that subjectivity are all of the problems that have plagued the assay for years and continue to do so in the clinical arena. Both CAMEO-4™ and MethoCult® assays require the investment of a tissue culture laboratory, including a quality microscope. HALO®-96 PCA^{EQ} also requires a cell culture laboratory and investment in the form of a plate luminometer. However, this instrument, together with the software that controls it, is a significantly lower investment than a good quality microscope. In addition, a well designed, instrument-based assay allows for calibration, standardization and validation in accordance with regulatory agencies [4].

There are also some other aspects that have not been addressed. A non-subjective, instrument-based assay does not require months of training and colony atlases to learn how to count and differentiate colonies. Since all HALO® assays measure cell proliferation, which cannot be measured using either CAMEO™-4 or any of the MethoCult® assays, the requirement to identify and count cell aggregates or colonies is obviated. As a result, HALO® assays can be used almost immediately out of the box. If the user knows how to accurately dispense reagents, they can use the assay. Furthermore, by virtue of its design, results obtained with HALO® can be compared over time within and between laboratories [13]. This allows for more meaningful proficiency testing. These aspects also save considerable time and money.

For these reasons, the long-term benefits, time and cost savings of implementing the HALO® Platform in a cell processing laboratory far outweigh the small cost of investing in an instrument and the minimal time required to learn how to use the assay.

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Changing the Paradigm

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About HemoGenix[®], Inc

HemoGenix[®] is a privately-held Contract Research Service and Assay Development Laboratory that produces and sells its services and assay kits in the U.S.A. and countries throughout the world. HemoGenix[®] specializes in developing predictive *in vitro* assay platforms for primary human and animal target cells. The assays have been specifically developed for Contract Research Services and as assay kits for in-house use by our customers. HemoGenix[®] is responsible for changing the paradigm and bringing stem cell hemotoxicity testing into the 21st century, by developing the HALO[®] Platforms that allow biotechnology and pharmaceutical companies to detect and predict the effects of large numbers of compounds on up to 14 different cell populations from 5 different species simultaneously. HemoGenix[®] is also changing the paradigm by providing standardized, instrument-based stem cell quality control and potency assays for transplantation and umbilical cord blood processing laboratories. HemoGenix[®] prides itself on bringing the best possible *in vitro* assay tools to its clients and customers.

TABLE 1
Comparison of Costs for HALO®-96 PCA^{EQ}, CAMEO™-4 and MethoCult® H4034 or H4434

	HALO®-96 PCA^{EQ} (Catalog No. K2-PCA1-1 or K2-PCA2-1)	CAMEO™ -4 (Catalog. No. KC-SC5-50H or KC-SC6-50H)	MethoCult® (Catalog. No. H4034 or 4434)
Price	\$520.00	\$495.00	\$394.00 (bulk) \$462.00 (tube)
No. of Samples/Replicates (bulk) No. of Samples/Replicates (tube)	24 samples @ 4 x 100µl replicates.	50 samples @ 4 x 100µl replicates	31 samples @ 2 x 1.1ml replicates 24 samples @ 2 x 1.1ml replicates
Cost/Sample:	\$21.67	\$9.90	\$12.71
What you get for the price (bulk): What you get for the price (tube):	1 x 15ml HALO-96 PCA ^{EQ} Master Mix 1 x 14ml Medium 1 x 96-well plate (sterile) 1 x 96-well plate (non-sterile) ATP standards ATP high and low controls ATP Monitoring reagent	1 x 38ml CAMEO-4 Master Mix 2 x 14 ml Medium 50 plates	1 x 100ml MethoCult reagent. 24 x 3ml MethoCult reagent.
Additional supplies:	Tubes, medium, pipette tips	Tubes, 1ml syringes, pipette tips.	Culture plates, needles, syringes, medium, tubes, pipette tips
Additional cost of supplies:	\$0.20	\$1.22	\$3.66
Instrumentation	Plate luminometer (\$10,000-\$15,000 including software)	Microscope (approx. \$20,000)	Microscope (approx. \$20,000)
Total reagent vol. required:	0.9ml	0.54ml	3ml
Total cell vol. required:	0.1ml	0.06ml	0.3ml
Total culture vol.	1ml	0.6ml	3.3ml
Total No. of cells required/sample:	20,000 for 4 replicates	20,000 for 4 replicates	30,000 for 2 replicates
Assay setup time/sample:	Approx. 10min/sample	Approx. 10min/sample	Approx. 10min/sample
Incubation time:	5, 6 or 7 days (flexible)	7 days or 14 days	14 days
Assay processing time:	30min/plate of 24 or 16 samples, including standards and controls.	Approx. 5min/4 replicate plate	5-10min read time/plate (min. 4hr for 24 samples)
Approx. labor costs for 1, 6, 12 and 24 samples:	\$13.33 / \$15.42 / \$17.92 / \$23.23	\$4.46 / \$26.75 / \$53.50 / \$107.00	\$15.00 / \$92.00 / \$184.00 / \$369.00
Total cost of performing the assay for 1, 6, 12 and 24 samples	\$33.46 / \$178.28 / \$351.55 / \$698.10	\$22.90 / \$137.35 / \$274.70 / \$549.41	\$33.00 / \$198.00 / \$396.00 / \$792.00 (bulk) \$36.00 / \$220.00 / \$440.00 / \$880.00 (tube)
Savings over MethoCult (bulk) Savings over MethoCult (tube)	-\$0.46 / \$19.72 / \$44.45 / \$93.90 \$2.54 / \$41.72 / \$88.45 / \$181.90	\$10.10 / \$60.65 / \$121.30 / \$242.59 \$13.10 / \$82.65 / \$165.30 / \$330.59	Not Applicable

TABLE 2
Comparison of Costs for HALO®-96 PCA^{EQ}, CAMEO™-4 and MethoCult® “Express”

	HALO®-96 PCA^{EQ} (Catalog No. K2-PCA1-1)	CAMEO™-4 (Catalog. No. KC-SC6-50H)	MethoCult® “Express”
Price	\$520.00	\$495.00	\$575.00 (bulk) \$748.00 (tube)
No. of Samples/Replicates (bulk) No. of Samples/Replicates (tube)	24 samples @ 4 x 100µl replicates.	50 samples @ 4 x 100µl replicates	31 samples @ 2 x 1.1ml replicates 24 samples @ 2 x 1.1ml replicates
Cost/Sample:	\$21.67	\$9.90	\$20.62 / \$31.17
What you get for the price (bulk): What you get for the price (tube):	1 x 15ml HALO-96 PCA ^{EQ} Master Mix 1 x 14ml Medium 1 x 96-well plate (sterile) 1 x 96-well plate (non-sterile) ATP standards ATP high and low controls ATP Monitoring reagent	1 x 38ml CAMEO-4 Master Mix 2 x 14ml Medium 50 plates	1 x 100ml MethoCult reagent. 24 x 3ml MethoCult reagent.
Additional supplies:	Tubes, medium, pipette tips	Tubes, 1ml syringes, pipette tips.	Culture plates, needles, syringes, medium, tubes, pipette tips
Additional cost of supplies:	\$0.20	\$1.22	\$3.66
Instrumentation	Plate luminometer (\$10,000-\$15,000 including software)	Microscope (approx. \$20,000)	Microscope (approx. \$20,000)
Total reagent vol. required:	0.9ml	0.54ml	3ml
Total cell vol. required:	0.1ml	0.06ml	0.3ml
Total culture vol.	1ml	0.6ml	3.3ml
Total No. of cells required/sample:	20,000 for 4 replicates	20,000 for 4 replicates	15,000
Assay setup time/sample:	Approx. 10min/sample	Approx. 10min/sample	Approx. 10min/sample
Incubation time:	5, 6 or 7 days (flexible)	7 days or 14 days	14 days
Assay processing time:	30min/plate of 24 or 16 samples, including standards and controls.	Approx. 5min/4 replicate plate	5-10min read time/plate (min. 4hr for 24 samples)
Approx. labor costs for 1, 6, 12 and 24 samples:	\$13.33 / \$15.42 / \$17.92 / \$23.23	\$4.46 / \$26.75 / \$53.50 / \$107.00	\$15.00 / \$92.00 / \$184.00 / \$369.00
Total cost of performing the assay for 1, 6, 12 and 24 samples	\$33.46 / \$178.28 / \$351.55 / \$698.10	\$22.90 / \$137.35 / \$274.70 / \$549.41	\$38.00 / \$228.00 / 457.00 / 914.00 (bulk) \$49.00 / \$292.00 / \$583.00 / \$1,167.00 (tube)
Savings over MethoCult (bulk) Savings over MethoCult (tube)	\$4.54 / \$49.72 / \$105.45 / \$215.90 \$15.54 / \$113.72 / \$231.45 / \$468.90	\$15.10 / \$90.65 / \$182.30 / \$364.59 \$26.10 / \$209.35 / \$308.30 / \$617.59	Not Applicable

TABLE 3
Percent Savings using CAMEO™-4 and HALO®-96 PCA^{EQ} over MethoCult® and MethoCult® “Express”

Percent Savings over:	HALO®-96 PCA ^{EQ}				CAMEO™-4			
	1 sample	6 samples	12 samples	24 samples	1 sample	6 samples	12 samples	24 samples
MethoCult® H4434/H4034 bulk reagent	0%	10%	11.2%	11.9%	30.6%	30.6%	30.6%	30.6%
MethoCult® H4434/H4034 tube reagent	7.1%	19.0%	20.1%	20.7%	36.4%	37.4%	37.6%	37.6%
MethoCult® “Express” bulk reagent	11.9%	21.8%	23.1%	23.6%	39.8%	39.8%	39.9%	39.9%
MethoCult® “Express” tube reagent	31.7%	39.0%	39.7%	40.2%	53.3%	53.0%	52.9%	52.9%

Please notice that the savings remains approx. the same for CAMEO™-4 regardless of the number of samples. This is because there is no additional time savings by counting colonies and therefore no additional cost savings.