Program No. 343

Using GemStone™ in the Routine Analysis of Clinical Stem Cell List-mode Data

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Introduction

CD34+ stem cell enumeration techniques for use in transplant programs vary substantially(1,2). Published ISHAGE standards allow for variations in analytical approach (3). ISHAGE Dual Platform and Single Platform Methods both use CD34, CD45, 7AAD, SSC, FSC to identify stem cells. Stem cells are then enumerated as a percent of viable intact white blood cells or by using beads spiked in the sample to obtain absolute counts of the stem cells as number per micro-liter.

In these manual methods a complex set of guidelines is used to construct a system of regions and gated two-parameter dot plots to isolate a very small sub-population of cells.

These manual techniques are fraught with potential errors in gate construction, region size and shape; terms for defining what constitutes a "positive" population are often unclear requiring operator interpretation. Coupled with inter-operator variability, standardization of stem cell enumeration remains a challenge.

Since there are two major approaches to stem cell enumeration in flow cytometer currently, single platform (flow cytometry only) and dual platform (flow cytometry plus hematology), we felt that we needed to take a look at both methods using the new automated modeling approach. Two GemStone Models were developed. One for the dual platform non-ISHAGE method and one for the ISHAGE single platform method that incorporated calculations of absolute stem cell count.

Fifty-eight (58) list-mode files (cord blood or bone marrow specimens) came from one facility using a single-platform method and fifty (50) list-mode files (peripheral blood, apheresis products, or bone marrow specimens) came from a facility using a dual-platform method.

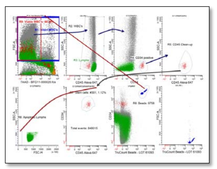
Goals

1. Reduce test variability
2. Reduce human error
3. Obtain results as good as human expert
4. Reduce tech time

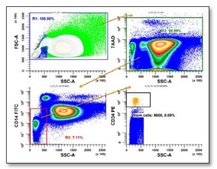
Current Methods

- Use complex gates
- Use manually defined regions
- Use manually positioned regions

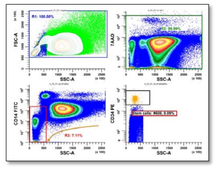
ISHAGE Method

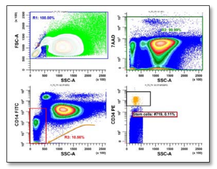


Non- ISHAGE Method



Region Placement Errors



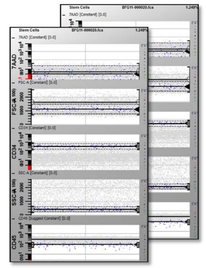


GemStone Analysis Methods

GemStone uses a patented Probability State Modeling (PSM) system to locate and classify populations.

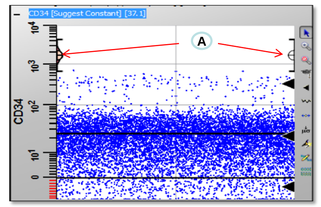
Parameter profiles are created for each marker relevant to the population of interest and grouped into a "Cell Type."

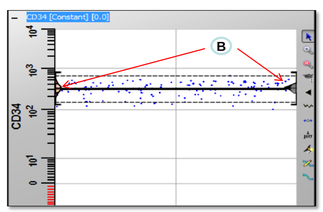
Then the PSM system automatically positions each parameter profile to select events belonging only to that cell type. Multiple cell types can be used in event classification.



A. Example parameter graph showing initial parameter profile position.

B. Example parameter graph showing parameter profile position after modeling process.





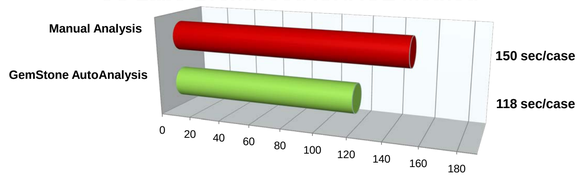
Notice only those events that meet the modeling classification remain selected.

Conditions such as relationships to other profiles can be added to the parameter profiles to enhance the event classification.

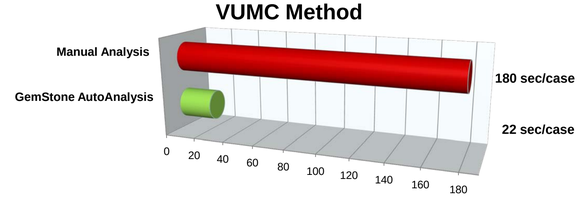
The result is a reusable model that can automatically process complex data files and enumerate stem cell populations.

Average Analysis Time per Case Manual vs. Automated

US Labs-Brentwood ISHAGE Method



VUMC Method



Manual vs. Automated Results

Automated enumeration of stem cells gave results equivalent to manual analysis by expert.

(Figure 1:ISHAGE Single Platform Method, Figure 2:Non-ISHAGE Dual Platform method).

Figure #1

#Stem Cells /ul

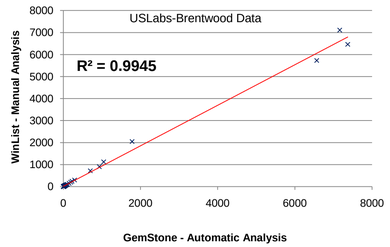
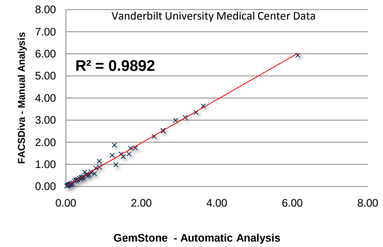


Figure #2

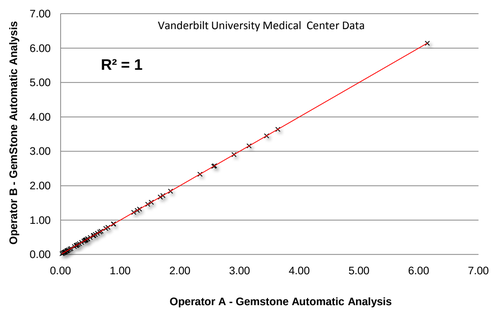
% Stem Cells in Viable Intact WBCs



Reproducibility

Multiple users in different laboratories get the exact same answers using GemStone (comparison of only two shown below).

% Stem Cells in Viable Intact WBCs- Multiple Operators



Conclusions

Stem cell analysis is still heavily dependent on human judgment and subjectivity. Ensuring that results are reproducible, accurate and precise still remains the biggest challenge in this assay.

We have shown that GemStone has the ability to answer this challenge with high correlation to current methods while significantly decreasing the technical time to process these files.

Additional model development to remove any remaining subjectivity in the automated processing is warranted and desirable.

Future work should also target the accuracy of stem cell enumeration using data sets with known population concentrations.

Discussion

Consensus on how stem cell enumeration is done has not been adopted in part because of the complexity of the gating required and uncertainty in region placement. With an automated system using a standard analysis model as we have shown here would remove that complexity and uncertainty. This standard modeling approach would also reduce human error, providing more reliable results to the clinical staff - thus improving patient care.

As shown by this study, even variations from the ISHAGE technique can benefit from using an automated standard model approach.

Expanding the number of cases and contributing laboratories as we continue model development, particularly those laboratories that currently use an absolute count technique, will be one of our goals as we move forward.

Persons interested in using the GemStone Standard ISHAGE Stem Cell Enumeration Model should contact Don Herbert at Verity Software House, PO Box 247, Topsham, ME 04086, or email: djh@vsh.com.

References

1."Stem Cell Panels: Acquisition and Analysis" Vanderbilt University Medical Center, Nashville, TN

2.Procedure for enumeration of CD34+ stem cells. USLabs, Brentwood, TN, (personal communication)

3.Current Methods for Identification of Hematopoietic Stem and Progenitor Cells in the Clinical Laboratory, Keeney, M. and Sutherland, R. , pp 321-340 Flow Cytometry in Clinical Diagnosis, 4th Ed Edited by Cary, J, McCoy, J, and Kern, D. , ASCP Press Chicago