Isolating Primary Cells 101: Which viability method is best for your research?

Ask the Experts Series #1

AllCells - Nexcelom Bioscience
Exclusive Webinar Event
2/13/2014

James Lee and Leo Chan, Ph.D.
Company Information

- Provider of image cytometry technology for cell concentration and viability
- Leo Chan, Ph.D.

- Provider of primary cells & bioservices for pre-clinical research
- James Lee
Important Questions to Address

• What is the process used for obtaining cells from bone marrow?

• What is the process used for obtaining cells from peripheral blood?

• What is the best and most accurate cell counting and viability method for primary cells?

• How do we select the appropriate method for viability measurement?
Key Points to Take Away

- AllCells key takeaways: decrease variability, increase speed and accuracy
- Nexcelom Bioscience key takeaways: the best method for concentration and viability measurement using different Cellometers, which is cell sample dependent
ALLCELLS is a leading B2B provider of hematology-based cells & services to the world’s life science research community for the past 16 years.

- **Products:** >800 healthy & diseased human tissue and primary cell products that enable drug discovery, stem cell research, and advancement of therapies

- **Bioservices:** Applications of hematology / immunology expertise; cell-based assay services and customized research studies

- **Core Competencies:** Hematology, immunology, human tissue procurement (healthy & diseased), large-scale cell isolations & characterization, stem cell expansion & differentiation

- **Operations:** Alameda, CA (HQ), IRB approved collection programs, Tissue Bank and Biologics Licenses
Primary Cells - Uses

- Drug Development – Pre-clinical Development
- Predictive Toxicology
- Stem Cell Research/Development
- Regenerative Medicine
- Medical Device Validation
Primary Human Cells by AllCells

- Normal Peripheral Blood -> MNC -> T Cells (untouched)
- Bone Marrow -> MNC -> CD34+
  (organ>isolation steps-> cell types)
  (Cell viability, counting methods suitable for each step)
Equipment Utilized

Miltenyi’s Separation Units

AutoMACS Pro Separator

CliniMACS Plus

CliniMACS Prodigy
Equipment Utilized

- Self-renewing totipotent stem cell
- Lymphoid precursor cells
- Myeloid precursor cells

Differentiating Cells

- Pre-T cell
- T cell
- Pre-B cell
- B cell
- Granulocyte precursor cell
- Monocyte
- Megakaryocyte
- Erythroblast
- Proerythroblast
- Reticulocyte

Differentiated Cells

- Activated T cell
- Activated B cell
- Neutrophil
- Basophil
- Eosinophil
- Macrophage
- Platelets
- RBC's
Whole Bone Marrow is drawn into a 10cc syringe containing heparin (80U/ml of BM) from the posterior iliac crest, 25ml/site, from a maximum of four sites.
Whole Bone Marrow

- Whole Bone Marrow -> CD34+ and CD133+ isolation methods
- Small/large scale isolations
- Fluorescence staining
  - Trypan blue method
  - AOPI method
- Cryopreservation Method

Post Thaw Data

<table>
<thead>
<tr>
<th>Date</th>
<th>Tissue Type/Unit</th>
<th>Lot Number</th>
<th>AO/PI Lot Number</th>
<th>Cellometer Count</th>
<th>Cellometer Viability</th>
<th>Hemacytometer Count</th>
<th>Trypan Blue Viability</th>
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<tbody>
<tr>
<td>11-20-13</td>
<td>ABM-CD34+ 0.5x10^6</td>
<td>BM4702</td>
<td>130904-01</td>
<td>0.8x10^6</td>
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<td>130904-01</td>
<td>0.3x10^6</td>
<td>92%</td>
<td>0.3x10^6</td>
<td>96%</td>
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</table>
Leuko Pak is PBMCs collected from donors by apheresis, from Ancient Greek ἀφαίρεσις (aphairesis, “a taking away”) is a medical technology in which the blood of a donor or patient is passed through an apparatus that separates out one particular constituent and returns the remainder to the circulation. It is thus an extracorporeal therapy. The apparatus we use is COBE Spectra Apheresis System manufactured by Caridian BCT. The separation of individual blood component is done with a specialized centrifuge. Spectra system may also be used to collect platelets, plasma and granulocytes.

Approximately three donor blood volume is processed and between 250-300ml of plasma and MNC is collected/donor.

• Average cell yield: $1.3-1.5 \times 10^{10}$
• Range: $0.9-3.5 \times 10^{10}$
• Lead Time: 2 weeks
Normal Peripheral Blood

- NPB -> T Cells (untouched) isolation methods
- -/+ selection
- Fluorescence staining
  - Trypan blue method
  - AOPI method
- Cellometer method

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<td>9.6x10^6</td>
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<td>10x10^6</td>
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<td>24x10^6</td>
<td>97%</td>
<td>22x10^6</td>
<td>91%</td>
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Custom Projects – AllCells Advantage

• Custom tissue collection
• Custom cell numbers
• Custom processing/packaging
• Custom aliquot and cryopreservation services
• Custom cell based assays
• Donor recruitment services
• Multiple cell types from single donor
• Donor-matched cells
• Mobilized collections
Custom Assay Bioservices

- **Flow Cytometry Services**
  - immunophenotyping
  - rare cell analysis
  - cell sorting

- **Custom Assays**
  - cell cycle analysis
  - apoptosis, phospho protein analysis
  - CD34+ determination under ISHAGE guidelines

- **Large-Scale Processing & Cell Isolation**
  - healthy or diseased human tissue
  - molecular products (RNA/DNA, etc.) & lysates

- **Drug Discovery Assay Services**
  - Luminex® multi-analyte assays
  - in vitro proliferation/inhibition assays (EC50)
  - in vitro expansion & HSC/progenitor differentiation
  - toxicology studies
  - colony formation assays e.g. CFU-GM, CFU-Mk
Introduction to Nexcelom Bioscience, LLC
Innovation and Expertise in the Science of Cell Counting

- 10 years – design, manufacture and market cell enumeration and analysis systems with image cytometry technologies
- Our approach: scientists help scientists to reduce error and increase assay accuracy
- 50% employees with science degree, 8 field application scientists in US
- Work with lead labs in each research field to develop products
- Spread best practice to other labs
What is a heterogeneous cell sample? How to select the right viability method?

Nexcelom Bioscience, LLC

Leo Chan, Ph.D. and Jean Qiu, Ph.D.
What is heterogeneous cell sample?

**Heterogeneous cell sample** contains target cells, other unwanted cell populations and tissue debris.
Develop better cell counting method for primary cells

- Dual fluorescence, nuclear acid binding AO/EB staining protocol was developed at Stanford, Prof. Leonard Herzenberg’s lab ([http://www.herzenberglab.org/Protocol-1/cell-preparation.html](http://www.herzenberglab.org/Protocol-1/cell-preparation.html))
- NIH/Vaccine Research Center (VRC) used AO/EB & manual counting
- Cellometer Vision automated AO/EB cell counting method for NIH/VRC since 2009
- Nexcelom optimized AO/PI staining as the standard protocol for primary cells
Dual fluorescence AO/PI viability assay

- AO is permeable to both live and dead cells
- AO binds to DNA and fluoresce bright green
- PI can only enter dead cells
  - Binds to DNA of the dead cells
  - Absorbs the green fluorescence of AO
  - Produces bright orange / red color
- No signal is generated from non-nucleated cells and debris
Leukapheresis samples:
over counting by bright field trypan blue method

Total cells: 119
Total FL cells: 39
Over counted cells: 80
They are not nucleated.
Over counting: 3x
The over counting ratio is sample dependent, not a systematic trend

- Manual counting using Hemacytometer and trypan blue
- Automatic cell counting using Cellometer Vision and AO/PI stains
- Experiment using Leukapheresis samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tbody>
<tr>
<td>Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(Manual counting # to Cellometer AO/PI #)</td>
<td>7X</td>
<td>5X</td>
<td>12X</td>
<td>3X</td>
<td>7X</td>
<td>5X</td>
</tr>
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</table>
Heterogeneous cell samples mostly used

- Mouse splenocytes
- Human Peripheral Blood Mononuclear Cells (PBMC)
- Human Mononuclear Cells (MNC)
- Human Leuko Pac
- Human Bone Marrow (BM)
- Human Cord Blood
- Mouse Tail Blood
- Bronchoalveolar lavage (BAL)
Mouse Splenocytes
Human PBMC

BR

AOPI
Human MNC
Human Bone Marrow
Human Cord Blood
Mouse Tail Blood
Using Trypan Blue Viability for Purified Primary Cells

AOPI stained all the CD34+ cells in BR
Which Cellometer is right for me?

• Trypan blue method is good for cell lines and purified primary cell samples
  - All Cellometer systems can perform Trypan Blue viability measurement
• AOPI method is good for primary heterogeneous cell samples, containing RBCs, platelets and cellular debris
  - Cellometer systems with dual FL can perform AOPI viability measurement
• Dual AOPI method was validated against total nucleated cell counting method by lysing the RBCs
Cell Counting Requirements

- Accuracy
- Consistency
- Speed
- Simplicity of interface (multiple users in lab)
- Minimal sample preparation
- Ability to capture and store data
- Create automated cell analysis reports
- Avoid contact with sample (non-aerosol)
- No fluidic system for potential clogging and contamination
Benefit to Cellometer users

- Call: 978-327-5340 and you will be connected to a technical support specialist
- Free in-lab training sessions (no limits)
- Free on-line support session via WebEx service
  Nexcelom technical support specialist can connect to any Cellometer via internet for training, trouble shooting and setting up new assays
- New in 2014: Monthly Cellometer Training Webinar
  For new lab members and new applications
Q&A

- Please use the prompt on the right of your screen to send a question to the organizer.
- Questions will be read and answered.
- Any offline questions may be sent to sales@allcells.com or sales@nexcelom.com.

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- Thank you!