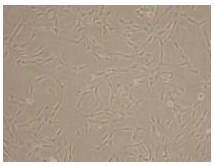
# Cell Culture Optimization

PACT Workshop: Translational Development & Scale Up:
Focus on Immune Cell Populations
Oct 26th – 27h, 2010
Darin Sumstad, CLS-Technical Lead


MSC's


Glioblastoma


UCB T-Regulatory Cells

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# Objectives

- Outline techniques utilized for successful technology transfer
- Discuss equipment used to make this process easier
- Discuss "lessons learned" and provide hints.
- Questions?

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# Technology Transfer

- Start with the Final Product
  - Packaging, Storage, and Shipping
    - Incorporate into experiment plans
    - Prepared reagent and product stability studies are often overlooked
    - Validate shipping processes and containers
    - Requirements for final product often impact production process flow

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# Technology Transfer

- Materials Compatibility
  - Can already stocked items be used for the culture?
    - Provide researcher with a spreadsheet template to complete.
    - Compare with available materials and re-distribute for acceptability approval
  - Not all materials will be available in-house
    - Complete appropriate documentation (Specifications, etc.)

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# Technology Transfer

## Materials Compatibility

**PACT Cells - Required Materials**
*Please complete as thoroughly as possible*

**Media Supplies:**

| Description | Vendor | Catalog # | Notes |
|---|---|---|---|
| DMEM | Gibco/Invitrogen | 12440-053 | |
| FBS | Hyclone | SH30070.03 | Using lot ARD26314 |
| L-glutamine 100x | Gibco/Invitrogen | 25030-081 | |
| 2-Mercaptoethanol | Gibco/Invitrogen | 21985-023 | |
| Gentamicin | Quality Biological | 120-098-031 | |
| | | | |
| Collagenase IV | Sigma | C1889 | |
| D-MEM/F-12 | Gibco/Invitrogen | 11330-057 | |
| | | | |
| Fibronectin | Becton Dickson | 356008 | Human natural |
| | | | |
| *Trypsin 0.05%* | *Gibco/Invitrogen* | *25300-054* | |
| *Versene* | *Gibco/Invitrogen* | *15040-66* | |
| *FBS* | *Gibco/Invitrogen* | *10010-023* | |

**Cultureware:**

| | | | |
|---|---|---|---|
| 10 cm Petri Dish | BD/Falcon | 353003 | |
| Sterile Serological Pipets | Sarstedt | 1, 5, 10, 25 mL | We can use what you have |
| 1.5 mL eppendorf tubes | | | sterilized |
| 50 mL Steriflip Filters | Millipore | SC6P00525 | 0.22 uM |
| 500 mL Sterile Filters | Nalgene | 566-0020 | 0.2 uM |
| *24-well Culture Plates* | *Falcon* | *353047* | |
| *75 cm ^2 Culture Flasks* | *Sarstedt* | *83.1813.002* | |
| *175 cm ^2 Culture Flasks* | *Sarstedt* | *83.1812.002* | |

**Other Equipment:**

| | |
|---|---|
| Small Iris Scissors (4) | sterilized |
| Small Forceps (sharp, no teeth - 4) | sterilized |
| #11 Scalpel and handle (2) | sterilized |
| | |
| *Hemacytometer* | |
| *10 uL Pipetman* | |
| *10 uL sterile filter tips* | |
| | |
| *Table Top Centrifuge* | |

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# Materials Compatibility

**PACT Cells - Materials Compatibility**

| | | | CellTherapy | | Research Lab | | |
|---|---|---|---|---|---|---|---|
| Media Supplies: | Specification | Description | Manufacturer | Man Catalog # | Manufacturer | Catalog # | Acceptable? |
| | CH38679 | IMDM | Gibco/Invitrogen | 12440-053 | Gibco | 12440-053 | ☑ |
| | CH100089 | FBS | Hyclone | SH30070.03IR | Hyclone | SH30070.03 | ☑ |
| | CH100014 | L-glutamine 100x | Gibco/Invitrogen | 25030-081 | Gibco/Invitrogen | 25030-081 | ☑ |
| | CH100134 | 2-Mercaptoethanol | Gibco/Invitrogen | 21985-023 | Gibco/Invitrogen | 21985-023 | ☑ |
| | CH100138 | Gentamicin | Quality Biological | 120-098-031 | Quality Biological | 120-098-031 | ☑ |
| | | | | | | | |
| | CH100139 | Collagenase IV | Sigma | C1889-50MG | Sigma | C1889 | ☑ |
| | CH100133 | D-MEM/F-12 | Gibco/Invitrogen | 11330-057 | Gibco/Invitrogen | 11330-057 | ☑ |
| | | | | | | | |
| | CH100140 | Fibronectin | Becton/Dickenson | 356008 | Becton Dickson | 356008 | ☑ |
| | CH100141 | Poly-D-Lysine | Sigma | P1024-10MG | | | ☑ |
| | CH100135 | *Trypsin 0.05%* | *Gibco/Invitrogen* | *25300-112* | *Gibco/Invitrogen* | *25300-054* | ☑ |
| | CH100136 | *Versene* | *Gibco/Invitrogen* | *15040-066* | *Gibco/Invitrogen* | *15040-66* | ☑ |
| | CH100137 | *FBS* | *Gibco/Invitrogen* | *10010-049* | *Gibco/Invitrogen* | *10010-023* | ☑ |
| | CH100807 | dH2O | Gibco/Invitrogen | 15230 | | | ☑ |
| | | | | | | | |
| Cultureware: | | | | | | | |
| | MA500355 | 100mm x 20mm TC Dish | BDFalcon | 353003 | BDFalcon | 353003 | ☑ |
| | MA501406 | 1 ml Serological Pipettes | BDFalcon | 356521 | | | ☑ |
| | MA500052 | 2 ml Serological Pipettes | Costar | 4021 | | | ☑ |
| | MA500053 | 5 ml Serological Pipettes | Costar | 4051 | | | ☑ |
| | MA500118 | 10 ml Serological Pipettes | Costar | 4488 | | | ☑ |
| | MA500123 | 25 ml Serological Pipettes | Costar | 4251 | | | ☑ |
| | MA500054 | 50 ml Serological Pipettes | Costar | 4501 | | | ☑ |
| | MA500919 | 1.5 ml microcentrifuge tubes | Fisher | 02681331 | | | ☑ |
| | MA500165 | 50 ml Conical Tube | BDFalcon | 352070 | | | ☑ |
| | MA137498 | 15 ml Conical Tube | Corning | 430052 | | | ☑ |
| | MA500356 | 50 mL Steriflip Filters | Millipore | SCGP00525 | Millipore | SC6P00525 | ☑ |
| | MA500353 | 150 ml Sterile Filter System | Nalgene (CX18497) | 565-0020 | | | ☑ |
| | MA500354 | 500 ml Sterile Filters System | Nalgene (CX18498) | 566-0020 | Nalgene | 566-0020 | ☑ |
| | MA500502 | 1000 ml Sterile Filters System | Nalgene | 567-0020 | | | ☑ |
| | MA500191 | *6-well Culture Plates* | BDFalcon | 3046 | | | ☑ |
| | MA500110 | *12-well Culture Plates* | Corning | 3513 | | | ☑ |
| | MA500495 | *24-well Culture Plates* | *BDFalcon* | *353047* | *Falcon* | *353047* | ☑ |
| | MA500805 | *25 cm^2 Culture Flasks* | Corning | 430372 | | | ☑ |
| | MA500065 | *75 cm^2 Culture Flasks* | Corning | 430641 | *Sarstedt* | *83.1813.002* | ☑ |
| | MA500229 | *175 cm^2 Culture Flasks* | Corning | 431080 | *Sarstedt* | *83.1812.002* | ☑ |
| | MA500216 | 250 ml Storage Bottle | Nalgene | 2019-0250 | | | ☑ |
| | MA500220 | 1000 ml Storage Bottle | Nalgene | 2019-1000 | | | ☑ |

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# Culture Conditions

- Seeding Density (cells/cm$^2$)
- Media Depth (x.x mm)
- Expected Growth Kinetics
- Proposed Quality Control

*Provide researcher with culture questionnaire and/or review pre-clinical notebook*

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# Culture Conditions

| Contact Information | Phone | Fax | Email |
|---|---|---|---|
| Primary | | | |
| Secondary | | | |

**Cell Line Information**

| Cell Type | Circle One | Subculture (Circle One) | |
|---|---|---|---|
| | Adherent | Trypsin | No Trypsin |
| | Suspension | Trypsin/EDTA | Other |

**Media Requirements (Complete formulation)**

| |
|---|
| |

**Culture Environment**

| |
|---|
| °C = |
| % $CO_2$ = |
| % $O_2$ = |

**Culture Parameters**

| |
|---|
| Seeding Density (cells/cm2)* |
| *If unknown - Total Cells / Vessel Size |
| Volume / Vessel (example: 4 ml/T25) |
| Passage Concentration/Confluence (%) |
| Seeding Concentration/Split Ratio |
| Days / Pass: |
| Passage Limit: |

Example Culture Questionnaire

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# Culture Conditions

- Seeding Density (cells/cm$^2$)
  - Most Pre-Clinical work done on a small scale
  - Must "Normalize" to allow for consistent culture conditions for clinical production
  - Example – PACT Cells:
    - Harvest ~ 430,000 TNC / TC6 1 well, Split 1:3
    - Calculate SD = 4.3E+05 / 28.8 cm$^2$ = 1.5E+04/cm$^2$
    - Data Review = ~3.0 – 5.8E+05 TNC/TC6 1 well
    - Range = 1.0 – 2.0E+04/cm$^2$

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# Culture Conditions

- Media Depth (mm)
  - Proper media depth is important for maintaining the culture environment and ensuring efficient gas exchange.
  - Example – PACT cells
    - Seeding = 1.5 ml's / TC6 well
    - Calculate MD = (Volume x 10) / Surface Area (cm$^2$)
    - MD = (1.5*10)/9.6= 1.6 mm
    - Volume for CF-5 = (3160 cm$^2$ x 1.6)/10 = 505.6 ml

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# Culture Conditions

*Flask Seeding Calculator*

| Enter the following information | | | |
|---|---|---|---|
| | Seeding Density | 1.00E+04 | /cm2 |
| | Media Depth | 1.6 | mm |

| CULTURE DISHES | | | |
|---|---|---|---|
| Dish Size | Surface Area (cm2) | Volume/Dish(ml) | TNC/Dish |
| 100mm x 20mm | 58.1 | 9.3 | 5.81E+05 |
| | | | |
| CULTURE PLATES | | | |
| Plate Size | Surface Area (cm2/well) | Volume/Well(ml) | TNC/Well |
| 96-Well | 0.32 | 0.051 | 3.20E+03 |
| 48-Well | 1.1 | 0.176 | 1.10E+04 |
| 24-Well | 1.9 | 0.304 | 1.90E+04 |
| 12-Well | 3.5 | 0.6 | 3.50E+04 |
| 6-Well | 9.6 | 1.5 | 9.60E+04 |
| CULTURE FLASKS | | | |
| Flask Size | Surface Area (cm2) | Volume/Flask(ml) | TNC/Flask |
| T-25 Upright | 12.5 | 2 | 1.25E+05 |
| T-25 | 25 | 4 | 2.50E+05 |
| T-75 Upright | 24 | 4 | 2.40E+05 |
| T-75 | 75 | 12 | 7.50E+05 |
| T-150 | 150 | 24 | 1.50E+06 |
| T-175 Upright | 44 | 7 | 4.40E+05 |
| T-175 | 175 | 28 | 1.75E+06 |
| T-185 | 185 | 30 | 1.85E+06 |
| CELL FACTORIES | | | |
| Factory Size | Surface Area (cm2) | Volume/Factory(ml) | TNC/Factory |
| CF-1 | 632 | 101 | 6.32E+06 |
| CF-2 | 1264 | 202 | 1.26E+07 |
| CF-4 | 2528 | 404 | 2.53E+07 |

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# Culture Conditions

- Culture Kinetics (Cell Doubling)
  - Growth rate is one of the best indicators of scale-up success and culture health
  - Baseline kinetics can be extrapolated from data provided by research lab
  - Example – PACT cells
    - 3.0 – 5.8E+05 cells / TC6 well
    - CD = (LN($TNC_{HARV}$/$TNC_{SEED}$))/LN(2)
    - $CD_{LOW}$ = (LN(3.0E+05/1.1E+05))/LN(2) = 1.45
    - $CD_{HIGH}$ = (LN(5.8E+05/1.1E+05))/LN(2) = 2.40

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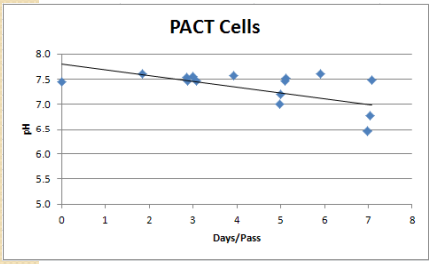
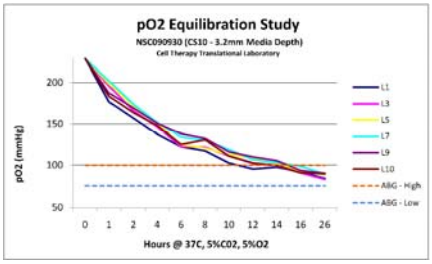
# Equipment

*BioProfile 400*

- BioProfile 400*
  - pH, $pO_2$, $pCO_2$, $Na^+$, $K^+$, Gluc, Lac, $NH_4^+$, Glu, Gln, Osm

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*Information obtained from Nova Biomedical

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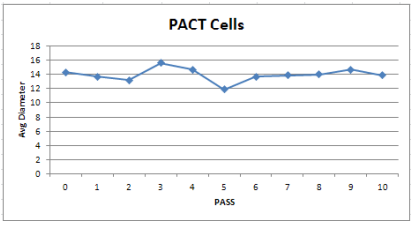
# Equipment

- Vi-Cell XR*
  - Automation of the standard trypan blue assay
  - % Viability
  - Total cell concentration
  - Total viable cell concentration
  - Mean cell size

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*Information obtained from Beckman Coulter

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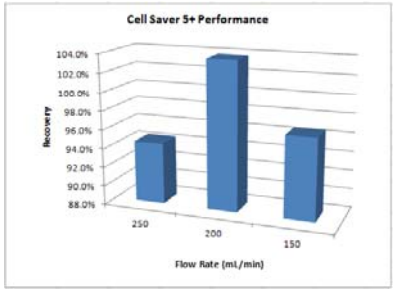
# Equipment

- CellSaver 5+ Autologous Blood Salvage System
  - 70, 125, and 225 mL bowl sizes
  - High Volume Concentration and Washing

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*Information obtained from Haemonetics

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# Quality Control

- Novelty of process sometimes require "unique" quality control assays
  - Equipment
  - Reagents
  - Technical Expertise
- Immunophenotyping
- Sterility
- Stability

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# REDUCTION OF NON-CLINICAL-/NON-CGMP-GRADE CULTURE REAGENTS AND MEASUREMENT OF RESIDUAL INGREDIENTS IN FINAL EARLY PHASE CELLULAR THERAPY PRODUCTS

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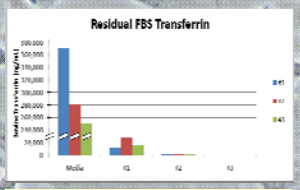
University of Minnesota Medical Center, Clinical Cell Therapy Laboratory [1], Minneapolis MN; University of Minnesota, Saint Paul[2], MN; University of Minnesota, Minneaplis[3], MN

## BACKGROUND

Novel cell therapies often require non-clinical- and/or non-cGMP-grade reagents for production. Realizing these limitations of early phase studies, the FDA may require that effort be made to reduce such reagents in the final product. We describe our approach to reduction and measurement of levels of residual culture medium in the setting of mesenchymal stromal cell production.

## MATERIALS & METHODS

Our current method of production includes culture in alpha-MEM supplemented with 16.5% fetal bovine serum and GlutaMAX. Following wash steps (310 xg for 5 minutes at room temperature) with 5% HSA, the product is sampled for final quality control testing before cryopreservation in PlasmaLyteA, 5% HSA, and 10% DMSO. To demonstrate reduction of culture reagents we performed three washes with 5% HSA, removing supernatent after each wash for testing of bovine transferrin levels [Bovine Transferrin ELISA Quantitation Kit, Bethyl Laboratories]. Bovine transferrin was selected over bovine albumin due to cross-reactivity with HSA. Conditioned medium served as the baseline control.




## RESULTS

See Figure 1. Substantial reduction of medium was accomplished with two washes (mean 290-fold reduction). An additional wash resulted in a mean 3451-fold reduction from baseline.

Figure 2. Typical absorbance microplate reader.. Using a bovine transferrin ELISA quantiation kit , sample dilutions were prepared in a 96-well microplate per documentation and read at 450nm.

## CONCLUSIONS

Reduction of certain reagents from final cell therapy products may be required by FDA. Cell processing may include a series of washes, and an indicator and method of measurement must be determined. Careful attention must be paid to any potential cross-reactivity of analytes. Here we demonstrated a successful approach using a bovine transferrin quantitative ELISA.

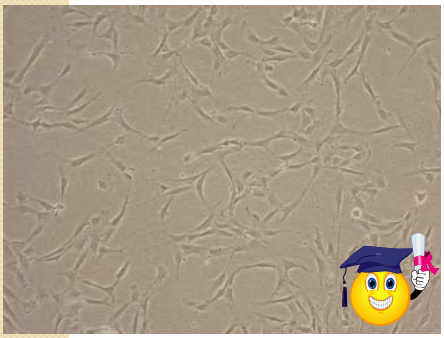
## ACKNOWLEDGEMENTS

We acknowledge the technical teams from the University of Minnesota Medical Center Clinical Cell Therapy Laboratory. Supported by the MN NHLBI-sponsored PACT contract.

# How do I know that my cells are happy?

Good Morphology = Happy Cells!

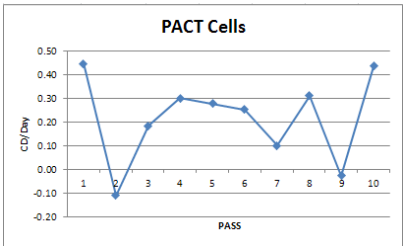
MSC Cells cultured with FBS



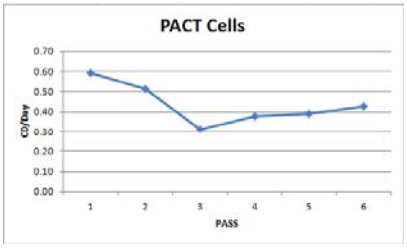
MSC Cells cultured with AB Serum

# Final Result - Optimized Procedure

**Initial cell culture protocol**




**Optimized cell culture protocol**

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# Important Considerations

- Medium (Supplements)
- Serum (lot variation)
- Temperature
- Gas ($CO_2$, $O_2$)
- Growth Surface
- Culture Technique

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# Process Map

- Review submitted process and develop a visual process map
  - Summarize key culture time points for group review
  - Visualization assists in predicting and avoiding "problems"

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## Lessons Learned

- Encourage open communication
  - Regular meetings and/or conference calls
- Direct Observation
  - Communicate with current processing staff
- Take a step back
  - Remember the complete process
- Expect hurdles
  - Always unexpected issues!

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## Cell Culture Optimization

Thanks for listening!

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**Anh Do**
**Lien Le**
**Jenny Kordosky**

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**Past and Present Collaborators**

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