



Invitation to Preview Asymmetrex®'s Online Rapid Stem Cell Counting

A New Landmark Biotechnology for Quantifying Many Different Tissue Stem Cell Types

Asymmetrex® recently developed and validated mathematical algorithms that can be used to determine the specific fraction or dosage of tissue stem cells in tissue cell preparations. Before this advance, the only method available for specific quantification of any tissue stem cell type was the SCID mouse repopulating cell (SRC) assay, which is limited to hematopoietic stem cells (HSCs). For a single HSC count, the SRC assay requires at least 30-40 expensive mice and 16 weeks. Asymmetrex®'s new online calculators give an instantaneous result after an input of simple 72-hour cell culture count data. Asymmetrex®'s kinetic stem cell (KSC) counting technology can quantify stem cells in any organ or tissue.¹⁻⁶

Online Rapid-Counting Calculator Portals

The first issued Asymmetrex® "RABBIT Count" algorithms for rapid quantification of tissue stem cells are now available in online calculator portals. After logging in, a user can enter 72-hour cell count data from evaluation cultures of CD34⁺ umbilical cord cells (UCB) or adipose-derived tissue cells to get the specific number of their respective HSCs or mesenchymal stem cells (MSCs). To calculate the stem cell-specific fraction or dosage of a sample, the calculators require only four basic inputs:

1. Tissue source
2. Number of days of culture of the sample since its tissue source's isolation
3. Cell culture medium used for the quantification analysis
4. Total cell number at 0 hours and at 72 hours of culture of the evaluated sample

How to Preview

Asymmetrex® is now inviting selected laboratories to preview a free online calculator portal with the two currently available calculators for quantifying CD34⁺ UCB HSCs or adipose MSCs.

Visit <https://asymmetrex.com/contact-us/> to request free access to a portal.

New calculators for other sources and types of tissue stem cells will be issued on a quarterly basis. Have a preference? Let us know when you sign up for a preview.

References

1. 2017 – US 9733236
2. 2019 – GB 2529921
3. Dutton *et al.* *OBM Transplantation* 2020;4:24; doi:10.21926/obm.transplant.2003117.
4. 2021 – No. PCT/US2021/63157208
5. Sherley *et al.* 2022, submitted.
6. See attached introductory slide deck.



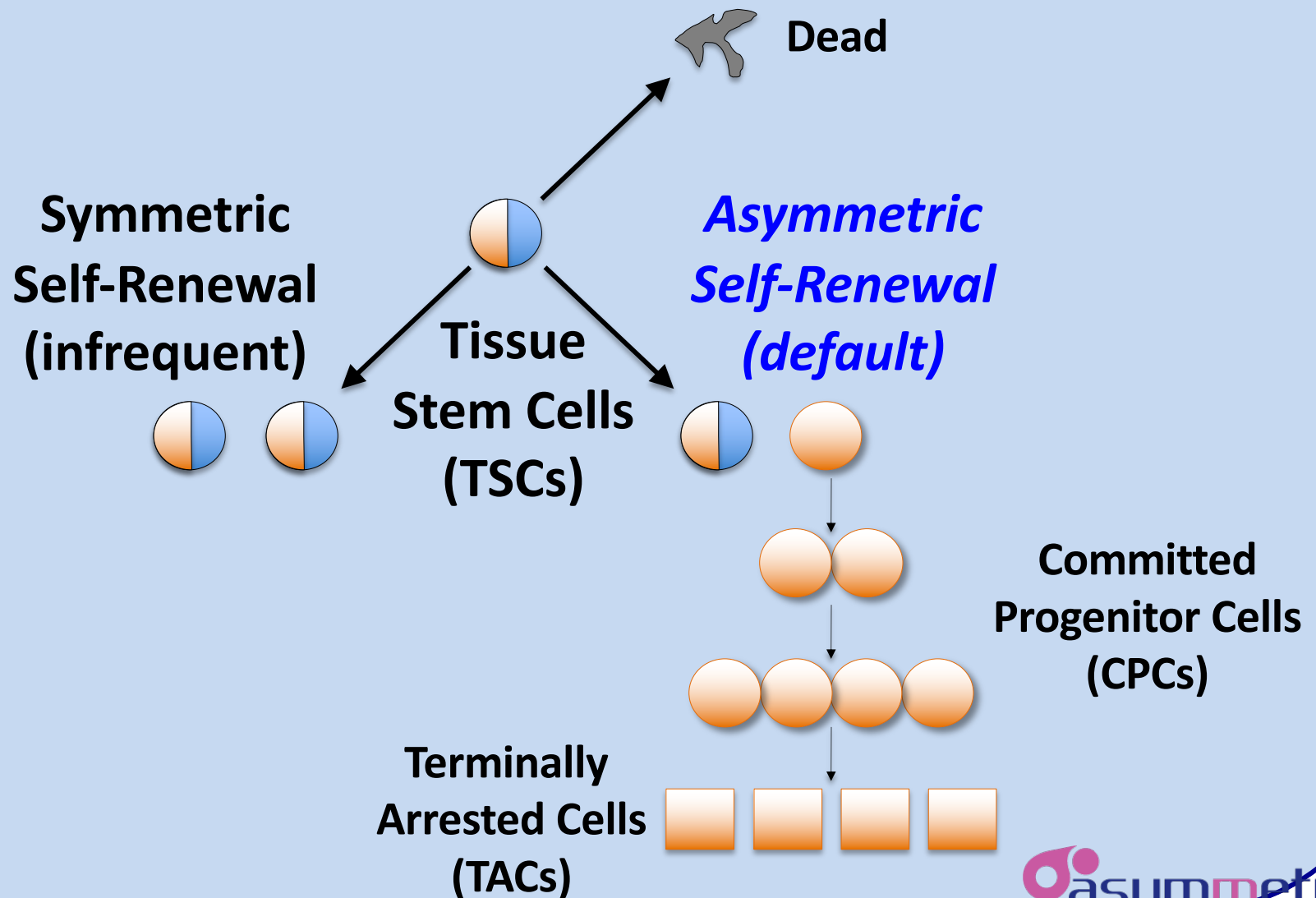
Kinetic Stem Cell (KSC) Counting:

A brief introduction to the technology

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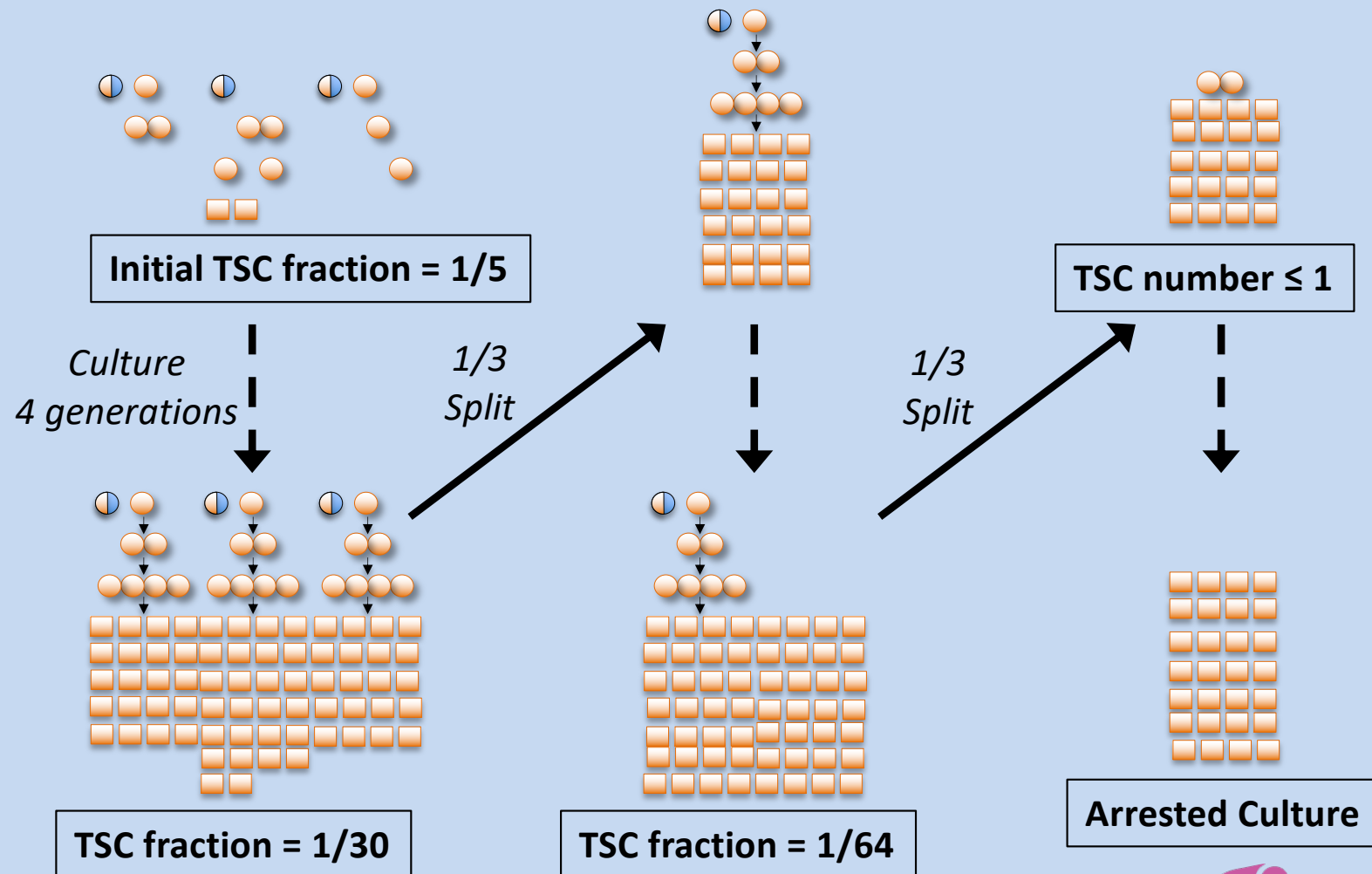
KSC Counting Principle I

In vivo TSC kinetics continue in cell culture.

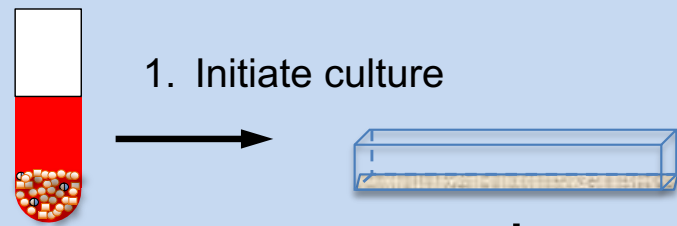


KSC Counting Principle II

Primary tissue cell cultures' total cell output depends on TSC-specific fraction and cell kinetics.



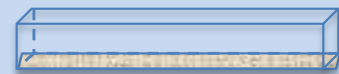
KSC Counting Begins with Serial Culture



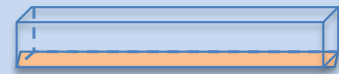
1. Initiate culture

Tested
Tissue Cells

(Adherent
or
Suspended)



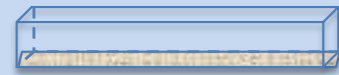
2. Culture



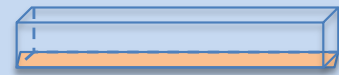
3. Count and passage



4. Culture

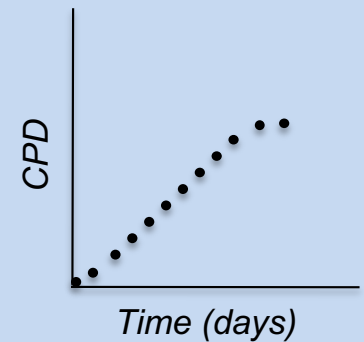


5. Repeat steps 3 and 4 until
no division or no cells detected



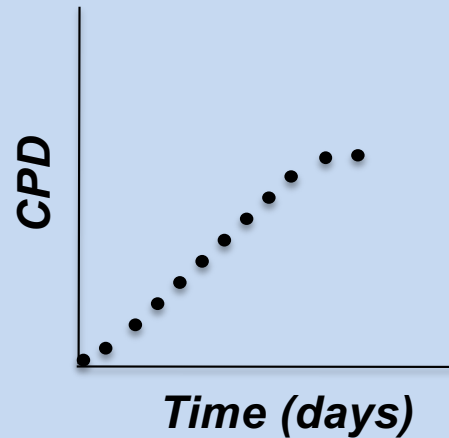
*A measure of the total
output of serial culture*

6. Transform cell
count data to CPD



**CPD,
cumulative population doublings**

CPD Kinetics Depend on Two Sets of Factors



- Rate
- Maximum
- Time of arrest

Known Culture Factors

Input cell number
 Split interval
 Split fraction
 Cell viability

Unknown Cell Kinetic Factors

TSC Number

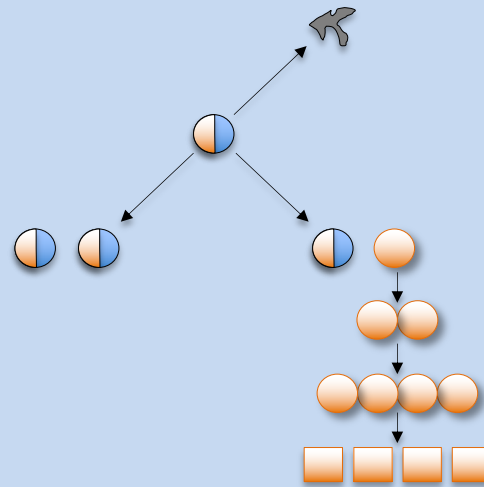
CPC Number
 TAC Number

TSC Viability
 CPC Viability
 TAC Viability

TSC Asymmetric CC Time
 TSC Symmetric CC Time
 CPC CC Time

TSC Symmetric Rate

CPC Division Number

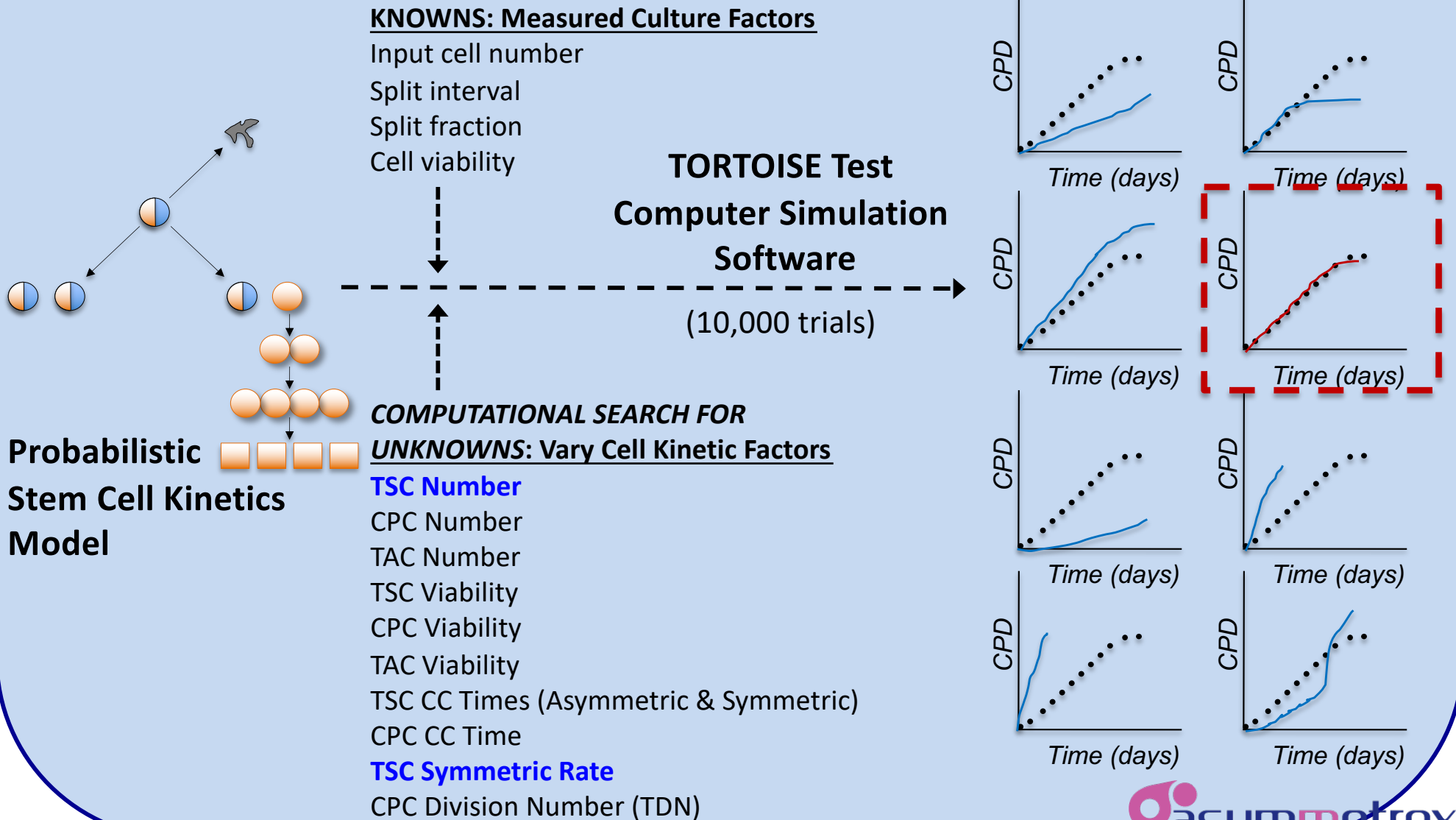


TSC, tissue stem cell; **CPC**, committed progenitor cell;

TAC, terminally-arrested cell; **CC**, cell cycle;

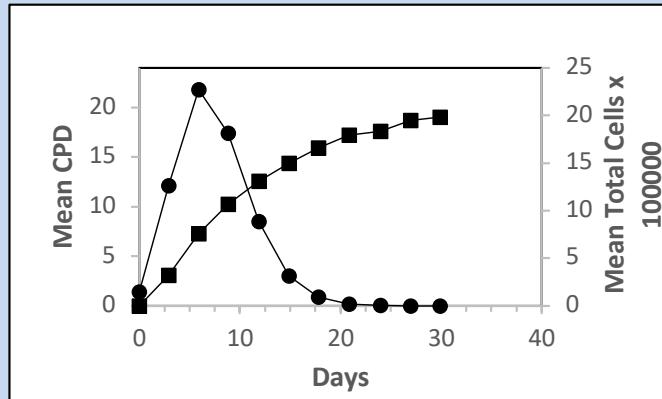
Division Number = number of divisions before producing TACs

KSC Counting – A computational simulation modeling approach



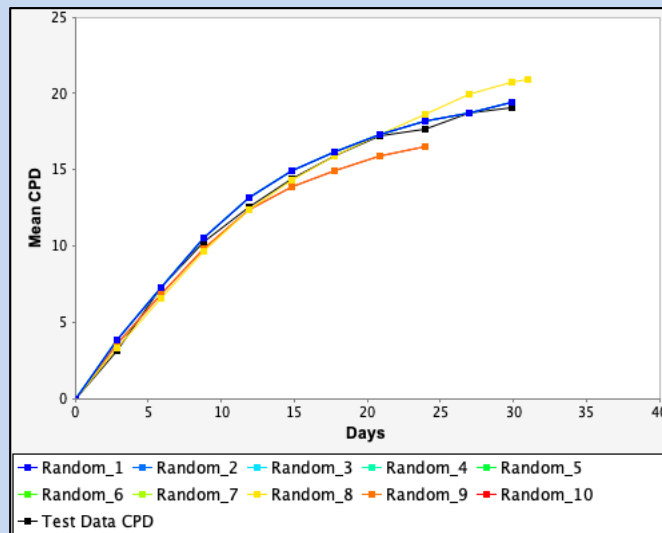
The KSC Counting Process

1.



Serial Cell Count Data

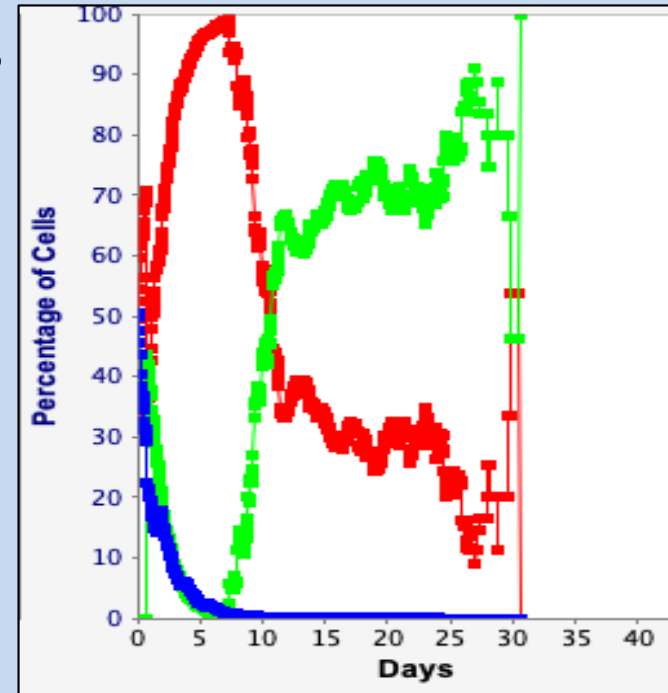
2.



TORTOISE Test Simulation

Example of 10 independent simulations

3.



KSC Counting Cell Subtype Kinetics

Blue – Tissue stem cells (TSCs)

Red – Committed progenitor cells (CPCs)

Green – Terminally arrested cells (TACs)

Note: Analysis for CD34⁺ UCB cells

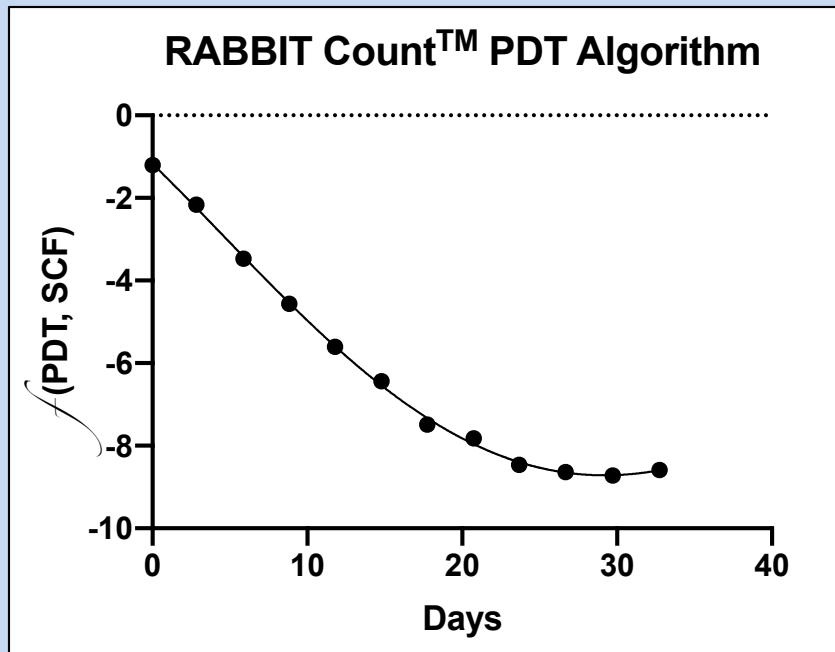
KSC Counting Validations

- I. Comparison to independent detection of asymmetric self-renewal divisions¹
- II. Analysis of effects of tissue stem cell-active agents (positive and negative factors)¹
- III. CD34⁺ fractionation analyses for HSCs^{1,2}
- IV. *Comparison to SCID mouse repopulating cell assays for HSCs²*

1. 2020. Dutton *et al.*, *OBM Transplantation* 4(3):24; doi:10.21926/obm.transplant.2003117.
2. 2022. Dutton *et al.*, submitted.

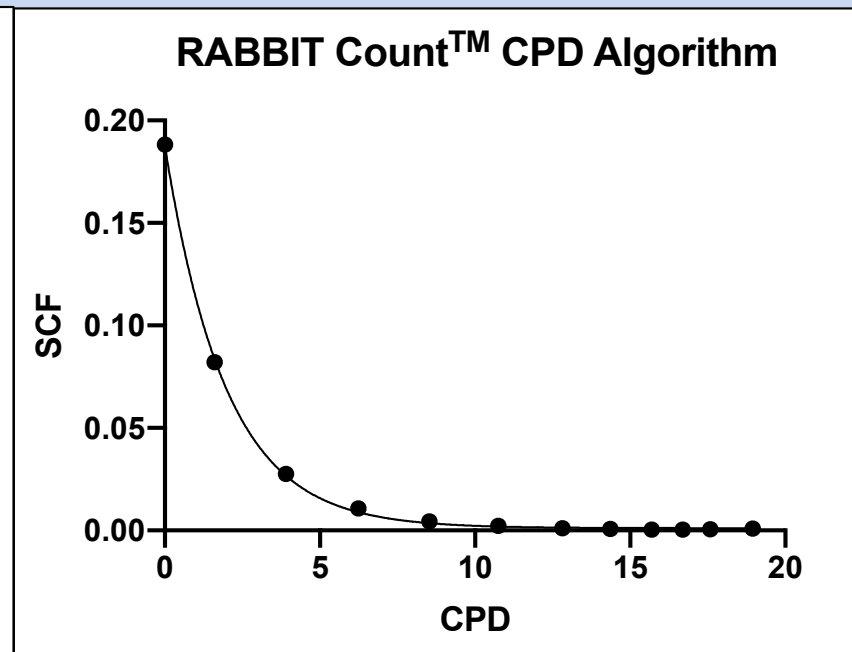
Rapid-Counting Algorithms

For any day of serial culture,
if you know the culture's PDT,



...you know the SCF.

For any future cell culture,
if you know the culture's CPD,



...you know the SCF.

Note: Data for CD34⁺ umbilical cord blood HSCs

SCF, stem cell-specific fraction; PDT, population doubling time;

CPD, cumulative population doublings

Example of Online Rapid-Counting Calculator Portal

✓ Result: 0.5607234854542854

Asymmetrex® Rabbit Algorithm For Human Adipose-Derived Mesenchymal Stem Cells

This calculator allows determination of the tissue stem cell-specific fraction (SCF) of primary human adipose-derived mesenchymal stem cells when cultured in the following commercial cell culture medium:

Thermo Fisher MesenPro RST™ Basal Medium supplemented with MesenPro RST™ Growth supplement (Kit Cat#1276012), 2 mM L-glutamine, and pen/strep.

Culture and counting procedures

1. In a 6-well plate, initiate evaluation cultures with ideally 50,000 to 100,000 total viable cells per 5.0 mLs of the culture medium specified above. Fewer cells can be used, but more cells should not be used. Recommend preparing 6 replicate wells.
2. Use 3 of the cultures to determine the mean total cell count 4-5 hours after plating. This analysis sets time = 0 hours.
3. Approximately 72 hours later, determine the mean total cell count in the remaining 3 cultures, time = "72 hours." This period should not be less than 66 hours and not more than 78 hours (i.e., range in fractional days from 2.75 to 3.25).

Note: Accurate tissue stem cell counting requires an increase in total cell number during the period of this analysis.

Calculator Procedure

1. Enter, **d**, the number days of serial culture of the Condition 4 cells at the time of their removal for SCF determination (e.g., 0, 3.0, 6.0). Entry of fractional days is recommended for increased accuracy.

d

2. Enter **N0**, the total number of cells (live and dead) in the evaluation cultures at time = 0.

N0

3. Enter **N72**, the total number cells (live and dead) after approximately 72 hours of culture.

N72

4. Enter, in units of fractional days, **T**, the actual period of time between the N0 cell count and the N72 cell count.

T

 Calculate

Human tissue stem cells counted to date

- Bone marrow hematopoietic stem cells¹
- Mobilized peripheral blood hematopoietic stem cells^{1,2}
- Umbilical cord blood hematopoietic stem cells*^{1,2}
- Umbilical cord tissue mesenchymal stem cells
- Bone marrow-derived mesenchymal stem cells
- Adipose-derived mesenchymal stem cells*
- Oral-derived mesenchymal stem cells
(bone, gingival, dental pulp)
- Liver hepatic stem cells
- Lung interstitial stem cells
- Corneal stem cells
- Amniotic membrane stem cells

**Available for preview now*

1. CD34⁺-selected
2. Unfractionated

KSC Counting Applications

- Quantity tissue stem cell-specific fraction in research studies
- Monitor tissue stem cell-specific fraction during expansion culture
- Optimize tissue stem cell fraction for biomanufacturing
- Certify the tissue stem cell fraction of manufactured products
- Certify the stability and viability of cryopreserved tissue stem cells *specifically*
- Certify the potency of tissue stem cell culture medium and growth factors
- Determine the tissue stem cell-specific dosage of treatment preparations
- Evaluate drug candidates for tissue stem cell-specific effects:
 - Positive – expansion factors; potential healing therapeutics
 - Negative – early preclinical identification of tissue stem cell toxicity that causes chronic organ failure

For additional information:

<https://asymmetrex.com/tortoise-test/>

<https://asymmetrex.com/rabbit-count/>

<https://asymmetrex.com/stem-cell-counting-center/>