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Comparison of Hemacytometer and HiRes Cell Counters Used in Potency Assays

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❑ **Background**

❑ **Data and Questions of Interest**

❑ **Results**

❑ **Summary**

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— Potency bioassays are used to measure the biological activity of a molecule.

— Bioassay performance depends on adding a target number of cells to the assay every time i.e. counting cells reproducibly.

— The current practice by most bioassay labs is to manually perform cell counting using a **Hemacytometer** to determine cell concentration and viability.

— Our bioassay lab recently acquired a new Roche Innovatis **HiRes** cell counter which is semi-automatic and reduces the time and labor considerably.

— A series of experiments using multiple cell lines was conducted to determine whether the HiRes could be a suitable alternative to counting cells using the Hemacytometer.

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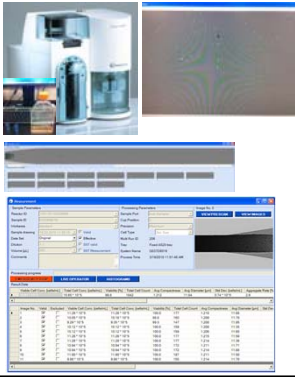

Hemacytometer

- Cells are harvested & loaded into the twin chambers A & B of the hemacytometer.

- The analyst counts the number of viable/live and non-viable/dead cells in quadrants 1, 2, 3 and 4 in each chamber.

- For count to be valid: the difference in the counts of two chambers A and B must be ≤10%; and each chamber must have ≥100 cells but ≤250 cells.

- The cell concentration is then calculated by adding the number of live cells in two chambers and adjusting for the dilution factor and number of quadrants.

- This method is labor-intensive, time-consuming and prone to high variability.

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Background
Two Instruments

Roche Innovatis HiRes

- Cells are harvested and 300 uL aliquot is put in a sample cup.

- The cup is loaded in the instrument, which adds the reagents and injects the sample into a chamber.

- 11 images are captured. Live & dead cells are counted for each image and the cell concentration is calculated.

- This method is semi-automated and fast.

Data
Experimental setup

- Variables
- *Cell Line* (1,...,11) (varying in size and type)
- *Target concentration* ($7x10^5$ cells/mL and $1.5x10^6$ cells/mL)
- *Method* (Hemacytometer and HiRes)
- *Analyst* (A, ...,T – 19 different analysts)
- *Replicate* (1,...,8)

Cell Line	1	2	3	4	5	6	7	8	9	10	11
Analyst	A, L	I, J	C, Q	F, O	H, N	B, E	F, O	G, T	R, S	F, P	D, K

- We created a new variable:

$$Prep = Cell\ Line\ by\ Analyst\ (22\ levels)$$

Prep describes the <u>preparation effect</u> when a specific analyst prepares a specific cell line. It is the sum of effects of the analyst and cell line; i.e. the variation of *Prep* is the sum of variations of analyst and cell line but we cannot estimate them individually due to confounding.
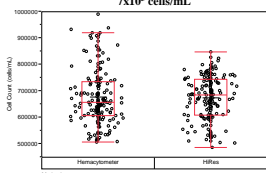
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Questions of Interest

Primary

1) To assess the agreement between two methods in measuring cell count.

2) To estimate loss of precision in reducing the number of replicates from eight (in this study) to two or three.
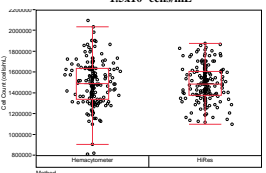
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Raw Data Plots
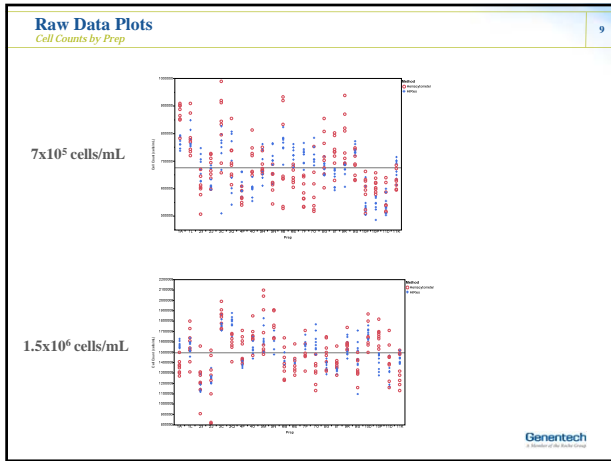*Cell Counts by Method at low and high concentration*

$7x10^5$ cells/mL

$1.5x10^6$ cells/mL

Method	Target	N	Min	Median	Max	Mean	Std Dev	CV
Hemacytometer	$7 x 10^5$ cells/mL	176	508,000	656,500	990,000	678,155	105,137	15.5%
HiRes	$7 x 10^5$ cells/mL	176	487,000	685000	849,000	675000	81,709	12.1%

Method	Target	N	Min	Median	Max	Mean	Std Dev	CV
Hemacytometer	$1.5 x 10^6$ cells/mL	176	814,000	1,495,000	2,300,000	1,495,722	213,866	14.3%
HiRes	$1.5 x 10^6$ cells/mL	176	1,100,000	1,490,000	1,880,000	1,495,625	173,125	11.6%

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Raw Data Plots
Cell Counts by Prep

7x10⁵ cells/mL

1.5x10⁶ cells/mL

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Results
Random Effects Model

Regression Model: To assess relationship between *Prep* and *Cell Count*, following random effects model was used (it was fit to each method and cell concentration separately),

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \qquad (1)$$

where,

Y_{ij} is the j-th ($j = 1,\ldots,r = 8$) observation in the i-th preparation ($i = 1,2,\ldots,22$)

μ is the mean cell count across the population of *Prep*s sampled

$\alpha_i \sim N(0, \sigma_{Prep}^2)$ is a random variable representing *Prep* effect (deviation of mean cell count for j-th *Prep* from the population mean); σ_{Prep}^2 represents the between-*Prep* variability.

$\varepsilon_{ij} \sim N(0, \sigma^2)$ is a random variable representing unexplained noise (deviation of cell count from the mean cell count for *Prep j*); σ^2 represents the within-*Prep* variability.

Then, coefficient of variation is calculated as,

$$CV(\%) = \frac{\sqrt{\hat{\sigma}_{Prep}^2 + \hat{\sigma}^2/r}}{\hat{\mu}} \times 100\% \qquad (2)$$

where values on the right hand side are estimated from the data.

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Method Comparison
CVs from the fitted regression model

Target	Hemacytometer	HiRes
7 x 10⁵ cells/mL	12.59%	10.52%
1.5 x 10⁶ cells/mL	11.59%	10.74%

- In each case, about 60-80% variation was explained by **Prep**.

HiRes is at least as good as Hemacytomter.

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Replicate Analysis
CVs from the regression model

- CVs are computed using the random effects model for different number of replicates

- For both target concentrations, the CV with $r = 2$ (~11%) was comparable to the CV obtained using $r = 8$ replicates; i.e. the gain in precision by increasing the number of replicates was minimal.

Target	Replicates	HiRes
7 x 10⁵ cells/mL	2	11.31%
	3	10.96%
	8	10.52%
1.5 x 10⁶ cells/mL	2	11.19%
	3	10.99%
	8	10.74%

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Sensitivity Analysis

A relative difference was calculated using the worst case values (min or max) for each *Prep*, where,

- *Old Reported Value = mean of 8 replicates for a given Prep*
- *New Reported Value = min or max of 8 replicates*
- *Relative Difference = 100% x (New Reported Value – Old Reported Value)/New Reported Value*

$7x10^5$ cells/mL $1.5x10^6$ cells/mL

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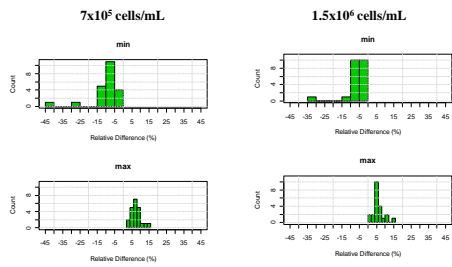
Summary of Results

- There is a good agreement between the manual Hemacytometer versus the automated HiRes cell counting method with percent difference <1% regardless of cell line.

- Loss of precision in reducing the number of replicates from eight to two was acceptable.

- HiRes is a suitable alternative to the current manual counting. It is more efficient and provides improvement in ergonomics.

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Acknowledgements

BT group
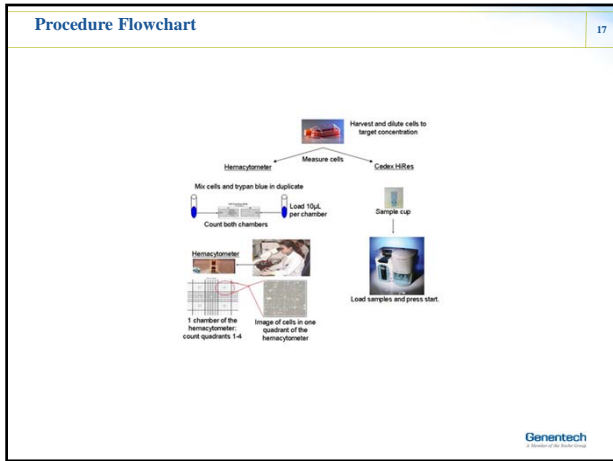- ❑ Ingrid Lesaca
- ❑ Melissa Woodrow
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- ❑ Imola Fodor

Thanks!

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BACKUP

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Procedure Flowchart

Raw Data Plot
Low Target

Raw Data Plot
Cell counts by Average Diameter

Suspension cells have smaller diameters

Adherent cells have larger diameters

Random Effects Model for Low Concentration
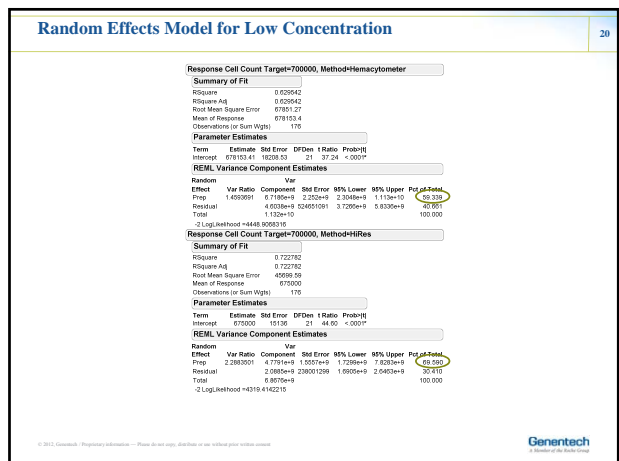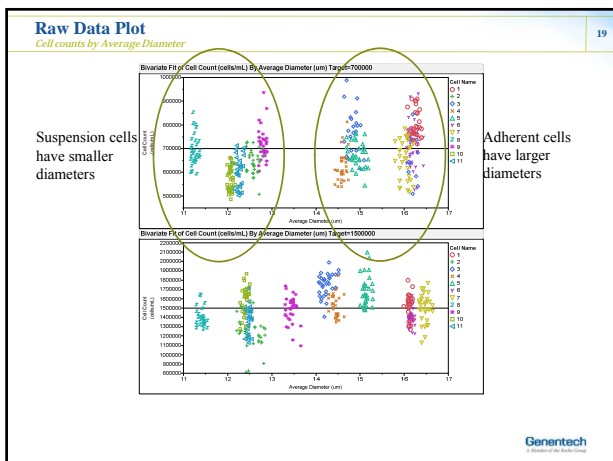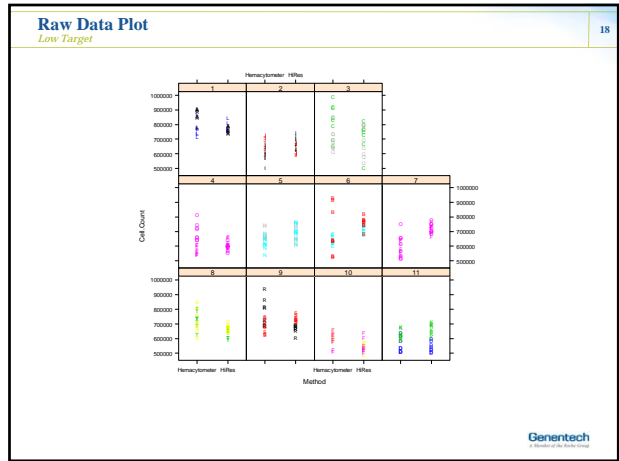
Random Effects Model for High Concentration