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Comparison of Manual Flask- Based vs. Cellomics Microplate-based Cytotoxicity Assay Methods

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Comparison of Manual Flask-Based vs. Cellomics Microplate-based

Cytotoxicity Assay Methods

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Introduction

Cellular toxicology assays use specific mammalian cell models and measures to evaluate cytotoxicity. In the pharmaceutical industry and elsewhere, counts of cells in flasks and trypan blue exclusion are widely utilized as part of assaying for compound-induced changes in cell growth and viability. From that answer, important decisions are made in setting of doses for subsequent studies in more complex systems. Issues of lack of sensitivity and reproducibility in the traditional method have left us looking for a reliable method to measure cell growth and cytotoxicity.

A cytotoxicity assay has been recently introduced that is sensitive and that offers increased efficiency in terms of sample handling and quantification. The changes in response measurements with this Cellomics® ArrayScan® II automated imaging system are consistent with profiles of known toxicants in the literature (Tencza and Sipe, 2004. J Appl Toxicol 24:371-7). Here, we measured cytotoxicity after exposing CHO cells to concentrations of a 3M compound that ranged from 10 to 800 µg/mL (24 hr). The assays employed were both the manual flask-based and the higher-throughput 96-well plate-based Cellomics methods.

Our results raise questions regarding the use of flasks and manual counts in reproducibly determining cytotoxic concentrations of novel chemical entities (NCE). We propose the need for both improved understanding regarding the rigor of trypan blue cytotoxicity assessments and the advantage of utilization of fluorescence microplate cytometers in cell-based assays.

Objective

To compare the sensitivity and reproducibility of selected methods used in evaluation of cytotoxicity

Materials & Methods

- CHO-K1 cells were seeded at equal densities in 96 well microplates and 25 cm² tissue culture flasks and incubated overnight at 37°C, 5% CO₂.
- Cells were then exposed to 3M-001 at 0 – 800 µg/mL for 20 hr at 37°C, 5% CO₂. DMSO concentration was maintained at 1% as per CRO methodology.
- Cells in microplates were stained with the fluorescent dyes Hoechst 33342 and YOYO-1 and interrogated using a Cellomics ArrayScan HCS Reader with on-the-fly analysis using the Multiparameter Cytotoxicity BioApplication. Cytotoxicity and Viability were calculated as demonstrated in Results.
- Cells in flasks were harvested by trypsinization. Counts and viability were determined using trypan blue exclusion.
- Cell counts and percent viability were measured at experiment's initiation (T₀) and at 20 hr after exposure to 3M-001 (T₂₀) to determine cell growth inhibition (see equations) relative to the vehicle control.

Cell Growth Calculations Using the Manual Flask-based and ArrayScan Measures

$$\text{Cell Growth} = \frac{(\text{Viable cells}_{\text{Treated } T_{20}} - \text{Viable cells}_{\text{Untreated } T_0})}{(\text{Viable cells}_{\text{Vehicle } T_{20}} - \text{Viable cells}_{\text{Untreated } T_0})} \times 100$$

T₀ = time of treatment, T₂₀ = 20 hours post treatment

ArrayScan Inputs:

- 1.) Cell Permeability Index records % cell death relative to control wells.
- 2.) Cell Counts sampled in well.

$$\% \text{ Viability} = 100 - \text{Cell Permeability Index} (\%)$$

$$\text{Viable Cells} = \text{Cell Counts} \times \% \text{ Viability}$$

Flask Inputs: Number of cells in flask based on standard calculation of number of cells excluding Trypan Blue in 4 hemacytometer fields

Example:

- Predose: 1,000,000 cells
- Vehicle control (20hr) cell number = 2,000,000
- Test Article (20 hr) cell number = 1,750,000 viable and 250,000 trypan blue-positive or Cell Permeability-positive cells (12.5%)

Calculation of Cell Growth in Flasks:

$$\frac{(1,750,000 - 1,000,000)}{(2,000,000 - 1,000,000)} \times 100 = 75\%$$

$$\text{Cell Growth Inhibition (CGI)} = 100\% - 75\% = 25\%$$

Calculation of Cell Growth in ArrayScan:

$$\% \text{ Viability} = (100\% - 12.5\%) = 87.5\%$$

$$\text{Viable Cells} = (2,000,000 \times 87.5\%) = 1,750,000$$

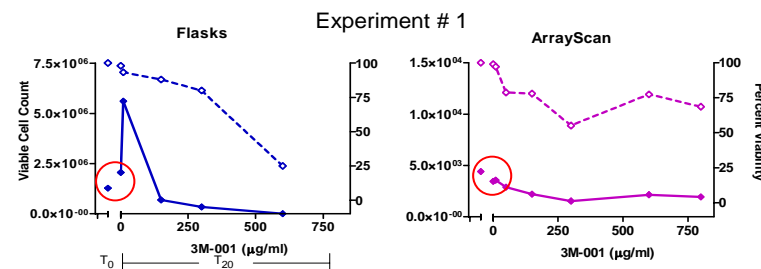
$$\frac{(1,750,000 - 1,000,000)}{(2,000,000 - 1,000,000)} \times 100 = 75\%$$

$$\text{CGI} = 100\% - 75\% = 25\%$$

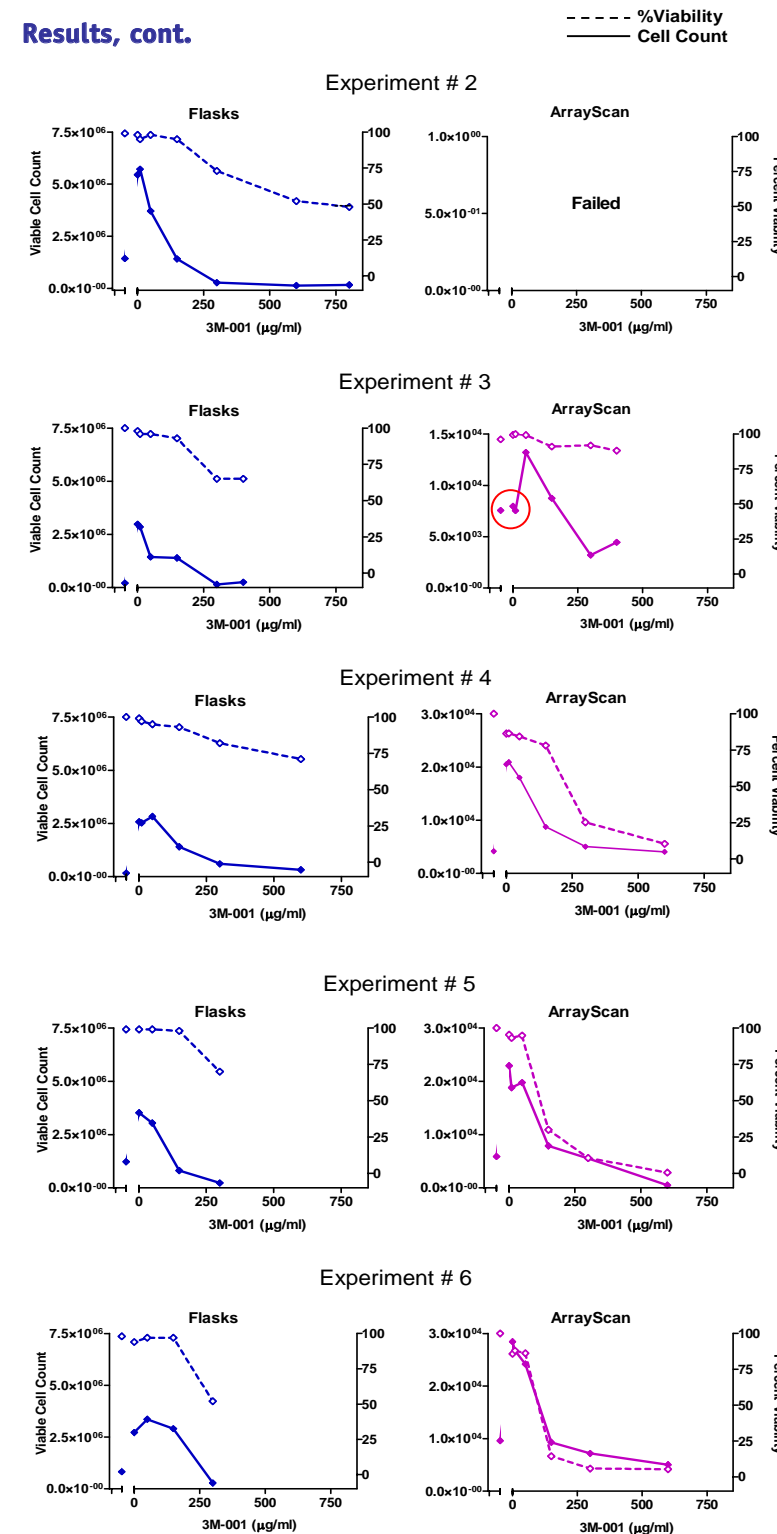
Results- Cell Counts and Viability

Dose response curves for CHO-K1 cells treated with 3M-001. Red circles indicate slow growing cultures from T₀ to T₂₀

--- %Viability
— Cell Count

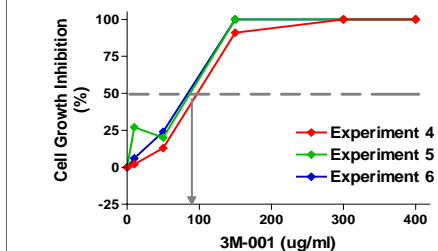


Results, cont.



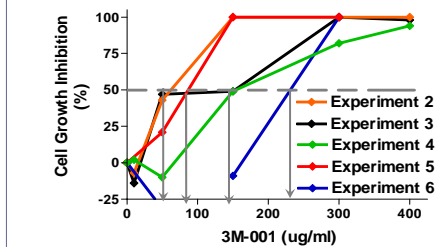
Results- Cell Growth Inhibition

ArrayScan results from cultures growing normally



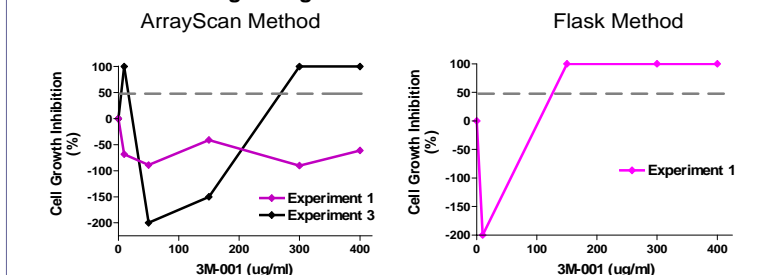
- Microplates and ArrayScan
- Very consistent CGI results in normally growing cultures
- IC₅₀ approximately 97µg/mL

Trypan Blue Exclusion (flask) results from cultures growing normally



- Flasks and Traditional Counts
- Inconsistent CGI results
- Relative to ArrayScan method, IC₅₀ lower, higher, or equal to ArrayScan
- Median IC₅₀ is ~50 µg/mL higher than ArrayScan.

Results from slow growing cultures



- Inconsistent and atypical CGI results in slow growing cultures

Conclusion

The ArrayScan delivers more consistent results than does manual counting of trypan blue exclusion in flasks

- In 5 traditional (flask) experiments, the Cell Growth Inhibition results did not replicate, despite normal growth rates (T₀-T₂₀) thus making sensitivity judgments difficult.
- There wasn't consensus in Cell Growth Inhibition between the flask and ArrayScan methods in the three parallel experiments in which cells had normal growth rates.
- In 3 microplate experiments, the Cell Growth Inhibition results of normal growing cultures (T₀-T₂₀) tested with ArrayScan did replicate.
- Our results raise questions regarding the current standard of flasks and trypan blue counting in determining a compound's cytotoxic level; both reproducibility and sensitivity are at issue.