Developing an Automated High-Throughput System for Cell Culture Process Development

Genentech
IN BUSINESS FOR LIFE

Louis Cheung, Jennifer Hu, Shun Luo*, Christina Bevilacqua, and Martin Gawlitzek
Late Stage Cell Culture, Process Research and Development
Genentech, Inc., South San Francisco, CA, USA

Presented at BioProcess International Conference, September 2008, Anaheim, CA

ABSTRACT

Cell culture process development is currently performed at different scales of shake flasks as well as bioreactors. The shake flask or bioreactor process is time consuming and labor intensive, and generates limited data per experiment. The current process is prone to human error due to repetitive pipetting and sampling of the shake flasks or bioreactors. Therefore, we developed an automated high-throughput system capable of handling suspension cell cultures to improve efficiency of cell culture process development. The Biomek™ FX, a high-throughput liquid handling system, was modified and integrated to prepare reagents and media in TubeSpin, a 50-mL conical tube that is utilized as a suspension cell culture vessel. High-throughput osmolality and pH measuring technologies were developed and integrated with the Biomek™ FX liquid handling system for automatic osmolality and pH adjustment during media preparation. The Quanta SC MPL, an automated high-throughput flow cytometer, was employed to monitor viable cell density, viability, as well as cell cycle profiles. Daily monitoring of the above mentioned parameters was performed to expand process knowledge. The automated high-throughput system is designed to set up and handle up to 384 TubeSpin cultures per experiment, an approximately ten times higher throughput compared to the conventional shake flask system. This technology can also be applied towards the improvement of clone screening, media development, as well as process optimization throughputs.

BACKGROUND

In cell culture process development (i.e. media development, process optimization), we currently rely on manual, labor intense low-throughput systems that include shake flasks & 2-liter bioreactors.

Limitations & Problems:
- Limited amount of data/information generated per FTE per experiment
  - Study range is too narrow
- Limited replicates
  - Data are less representative
  - Limited statistical analysis
- Ergonomic issues
  - Repetitive pipetting
  - Repetitive opening & closing of caps

Therefore, this poster highlights the development and implementation of a High-Throughput System for Cell Culture (HTS-CC) that will enable us to have a ≥ 10x increase in throughput compared to our traditional systems.

INTRODUCTION OF A HIGH-THROUGHPUT SYSTEM

Primary Considerations:

I: Improve efficiency and throughput (96-well format) while maintaining equivalence to large-scale agitated cell culture system
II: Existing automated liquid handling technology in SBS compliance:
- a: Reliable lab automation technology
- b: Benefit from high throughput analytical tools

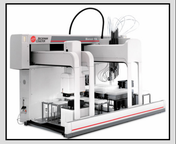
I: Maintaining equivalence:
- Cell culture vessel
  - TPP Tubes (50-mL)
  - Agitated suspension cell culture

IIa: Reliable lab automation technology in SBS compliance:
- Liquid handling system
  - Biomek FX^P (Beckman Coulter)

IIb: Compatible high throughput analytical tools:
- Quanta SC MPL (Beckman Coulter)
- Model 20G Osmometer (Advanced Instruments)
- SpectraMax M2e (Molecular Devices)

OVERVIEW – Liquid Handling System

Beckman Coulter Biomek FX^P
- Dual-arm configuration
  - 96-Multi-Channel pipettor
  - Span-8 pipettor
    - ➢Bulk-dispensing capability
    - ➢Enables the system to work with a variety of sample racks & sample vessels including tubes, vials, and plates



Customized 96-tube Rack
- 12 x 8 configuration
- Fully customized to fit on the Biomek FX^P platform
- Allow direct transfer of rack from Biomek FX^P into incubator

Customized 50-mL Culture Vessel
- Vented cap with septum
  - Vents: 0.22 µm filter
  - Septum: pre-slit silicone piece

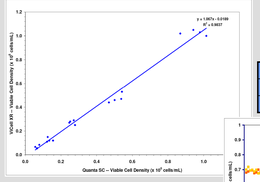
- Allow direct insertion of Span-8 probes
  - Reagent dispensing and mixing
  - Daily sampling

Customized Sterile Bulk-Dispensing Unit
- Minimize manual handling and possible contamination
- Multiple liquids handling capability

OVERVIEW – High Throughput Analytical Tools

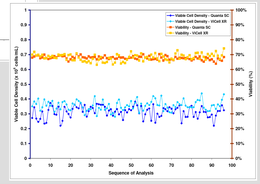
Beckman Coulter Quanta SC MPL
- Flow Cytometry
  - Electronic volume for accurate sizing
  - Light source: 488 nm Laser & UV Arc Lamp
  - Three color fluorescence detection
  - Side scatter for granularity measurements
  - Multi-Plate Loader (MPL) enables experiment setup in 96-well high-throughput format

Measurements:
- Cell Counts – Based on Electronic Volume
- Viability – Based on Propidium Iodide (PI) staining



Viable Cell Density (cells/mL) Measurement, N=96

	Quanta SC MPL	Vi-Cell XR
Mean*	0.31 x 10^6	0.36 x 10^6
SD	0.04	0.03
CV	12.3%	9.3%

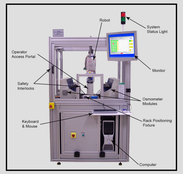


Viability Measurement, N=96

	Quanta SC MPL	Vi-Cell XR
Mean*	67.8%	68.1%
SD	1.2%	2.3%
CV	1.8%	3.4%

OVERVIEW – High Throughput Analytical Tools (cont')

Model 20G Osmometer
- From Concept to Delivery in 6 Months
- Measures 96 samples in 30 min vs 320 min using a conventional osmometer



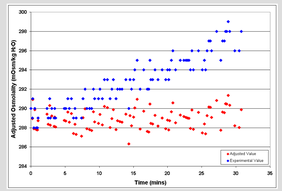
Specifications:
- Sample volume: 20 µL
- 96-well format: 12 x 8 layout
- Throughput: 96 samples in 31 minutes
- Measuring Range 50 to 850 mOsm/kg H_2O with error range of plus or minus 1%
- Data output compatible to Biomek FX^P software

Analysis of Osmolality in 96-well Format

Problem: Osmolality possesses a gradual upward trend during the 96-well format analysis due to evaporation

Adjust the experimental value using the correction constant whereas,

Correction Constant
= Slope of the Plot (% increase of osmolality versus time)
= 0.0885 % increase of osmolality per minute



Analysis of 290 mOsm/kg H_2O Standard Solution in 96-well Format (after adjustment):	
Average (N = 98)	288.9 mOsm/kg H_2O
Standard Deviation	1.0 mOsm/kg H_2O
CV	0.4%

SpectraMax M2 spectrophotometer
- 96-well format
- Multi-detection capability
- Utilize HydroPlates technology to measure pH in microplates
  - pH range: 5.0 to 8.0
  - Resolution: up to 0.01 pH
- Preliminary study of pH measurement using standard solution:

pH	5.0	6.0	7.0	8.0
Averaged	5.12	6.10	6.98	8.20
Standard Deviation	0.05	0.03	0.03	0.04
%CV	1.03	0.47	0.47	0.49

- Comparability of pH measurements:

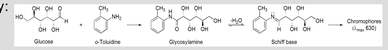
	HydroPlate	Nova 400	pH meter
Measurement of pH for Genentech in-house media	7.06	7.12	7.21

Current Monitoring Capabilities

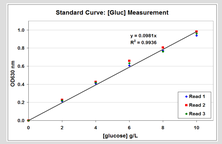
Parameters	Analytical Tools	Chemistry/Method	Sample Volume (µL)	Throughput (mins/96 samples)
Cell Count & Viability	Quanta SC MPL	Propidium Iodide Staining	20 – 30	~ 90
Cell Cycle		NIM-DAPI	100	~ 90
Osmolality	20G Osmometer	Freezing Point Depression	20	~ 32
pH	SpectraMax M2e	PreSens Hydroplates	200	~ 30
Glucose		O-toluidine	100	~ 20
Lactate		Lactate dehydrogenase	100	~ 20

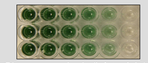
Current Monitoring Capabilities

Glucose Measurement
- Fluorescence-based: detects glycosylamine formation by measuring the increase in absorbance at λ = 630nm
- Chemistry:



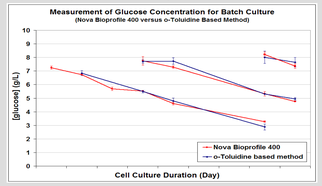
- Standard Curve:



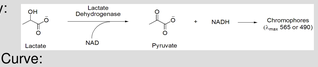


Glucose standards (left to right)
[glucose] = 10, 8, 6, 4, 2, 0 g/L

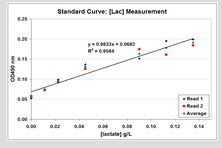
- Comparability with Nova Bioprofile 400 (BGA):



Lactate Measurement
- Fluorescence-based: detects NADH formed from oxidation of lactate at λ = 490nm
- Chemistry:

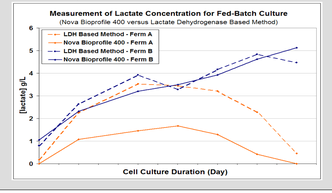


- Standard Curve:



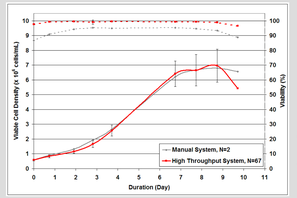
Lactate standards (left to right)
[lactate] = 0.225, 0.18, 0.135, 0.1125, 0.09, 0.0425, 0.0225, 0 g/L

- Comparability with Nova Bioprofile 400 (BGA):



RESULTS – Automated (HTS-CC) versus Manual System

Comparison of cell growth and viability in 50-mL tubes
- **Single Condition**



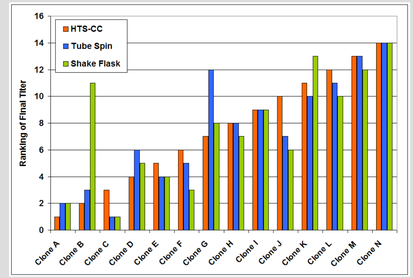
Detection Method:
- ViCell for Manual System
- Quanta SC MPL for Automated System

- Cells were able to reach similar peak cell density at ~ day 7 to 8.
- Similar trend of viability was also observed.

RESULTS – Automated (HTS-CC) versus Manual System (cont')

Clone Evaluation Experiment
- Total clones: 14
- Duration: 14 days
- Culture conditions & feed strategy: follow Genentech standard clone evaluation protocol
- System to be evaluated:
  - HTS-CC (N=4)
  - Tube Spin – Manually Setup (N=1)
  - Shake Flask – Manually Setup (N=1)

Ranking of Final (Day 14) Titer



Results indicated that the HTS-CC was able to generate similar ranking in terms of final titer as comparing to the manual system (i.e. shake flasks & tube spins).

SUMMARY

- Successfully developed a high-throughput system for cell culture (HTS-CC) with ≥10x throughput compared to manual operation:
  - Current maximal throughput: 384 samples (4 x 96 samples)
  - Traditional manual throughput (shake flask) : 30-40 samples/FTE

- High-throughput system has been successfully implemented for routine usage within department:
  - Media development
  - Clone evaluation
  - Process development

- Development of database to ease data management and analysis is underway

ACKNOWLEDGEMENTS

Shun Luo*

Genentech
- Bob Kiss
- Brian Kelley
- Dana Andersen
- Ann Lee
- John Stults
- Natalie Walker

Advanced Instruments

Beckman Coulter

Optimum Bioprocessing
- Pete Florez

ACME Automation
- Jeff Cahlik

Wynn Works
- William Wynn