Thermo Scientific HyCell CHO Medium: Targeted for high cell density and productivity across a broad variety of CHO clones

May/June 2013

Agenda



- Introduction
- Thermo Scientific™ HyCell™ CHO medium development
- Conclusion

Thermo Scientific HyCell CHO Medium Development

Broad spectrum of CHO cell types and variants

Goal:

- Improve protein production, achieve higher cell densities, and suitability for use by a broad spectrum of CHO cell types and variants
- Utilize high-throughput screening for much larger design space and a higher degree of replication for each treatment
- Further optimization
  - Shake-scale design of experiment (DoE) design
- Final formulation confirmation in shake
- Verification at scale in bioreactor

Comparability between screening platforms

Validation of high-throughput methodology:

- Evaluate productivity trends between shake and plate
- Six proprietary CHO cell lines were used in both plate and shake studies
- Exceptionally similar trends between shake flask and plate level studies
- Large-scale screening efforts:
  - 10 x 4 factor level screening of a media component
  - Ten concentration levels were tested using four different CHO cell lines

Versatile performance across a variety of CHO clones

High-throughput Screening (HTS)

96-Deep-well Plate Studies

- Deep-well plates were prepared for media addition, mixtures, component variation, and cell seeding using a Hamilton MICROLAB™ STARlet liquid handling system
- A total culture volume of 1.1mL was used, seeding densities were 0.25 x 10^6 viable cells per mL, with either six or eight replicates for each condition, depending on the study
- Samples were taken daily after day-3 for viable cell density counting, using a Roche Cellavista™ cell imaging system
- Additional samples were taken later in culture for productivity analysis until viability dropped below 50%
  - Octet™ QK 384 system for IgG producers and Matched-Pair Antibody Set for human tPA ELISA from Enzyme Research Laboratories

HTS for much larger design space and higher degree of replication

Shake Flask Studies

Shake Flask Studies

- Duplicate 50mL cultures for each condition
- Seeded with 0.25 x 10^6 viable cells per mL in 125mL shake flasks
- Straight batch culture format was used in all shake flask studies and cultures were incubated at 37°C, 5% CO_2 with constant agitation
- Daily samples were taken, beginning day-3 to assess viable cell density, using a Beckman Coulter Vi-Cell™ cell viability analyzer
- Additional samples were taken later in culture for productivity analysis until viability dropped below 50%
  - Octet QK 384 system for IgG producers, and Matched-Pair Antibody Set for human tPA ELISA from Enzyme Research Laboratories

Exceptionally similar trends between HTS studies and shake flask

Statistical Analysis

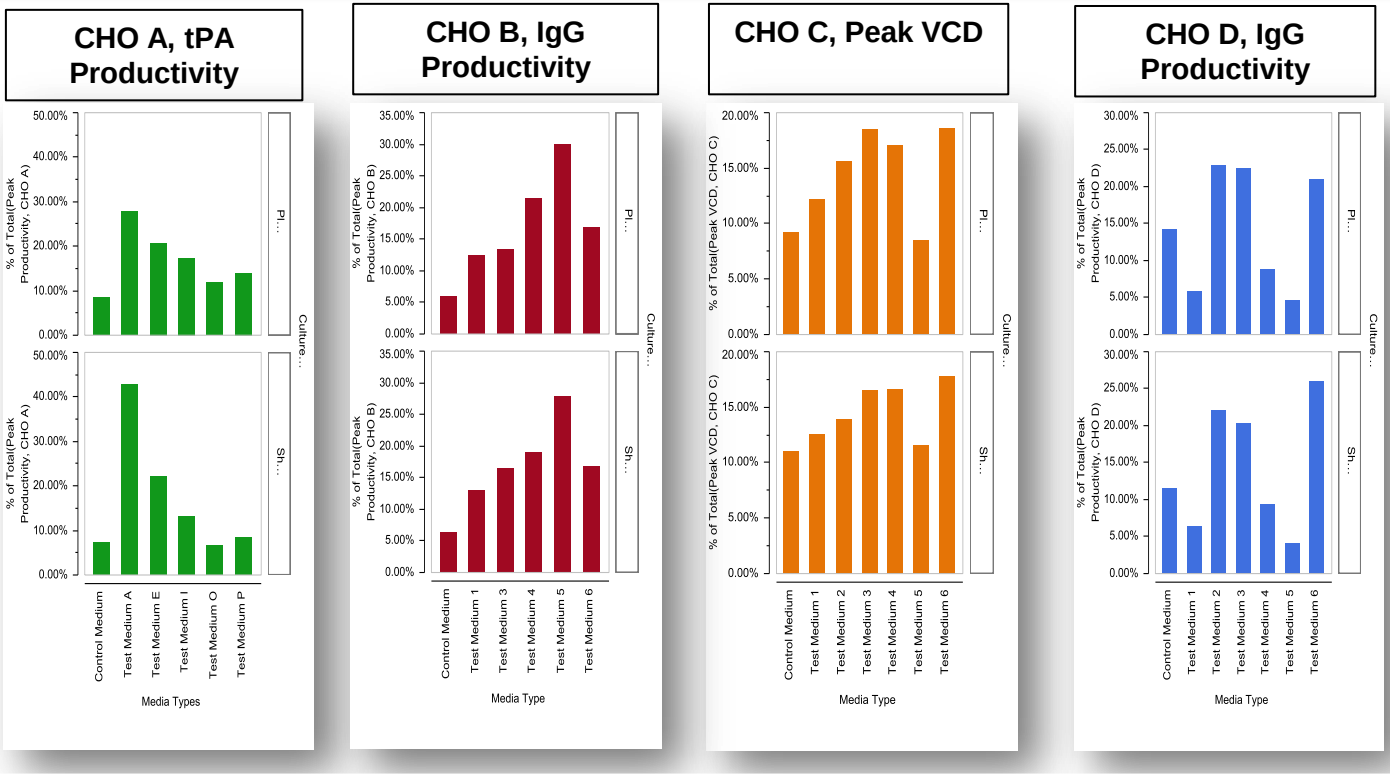
Statistical Analysis and DoE Studies

- Average (n=6), percent of total, peak viable cell densities (VCD) and productivities were tabulated and graphed using SAS Institute's JMP™ 9 software for method comparison studies
- One way ANOVAs were calculated for the component concentration study, comparing all replicates (n=8) of each of the 10 levels for each of the four cell lines using a standard least squares model. Seeded with 0.25 x 10^6 viable cells per mL in 125mL shake flasks
- Where preferences for concentration levels were significant, post-hoc Tukey HSD tests were computed to reveal the best level to use in new media formulations
- A simplex lattice mixture design, using optimal formulations (OF) 1, 2 and 3, was produced and analyzed using JMP software, as were ternary plots for peak VCD and peak productivity for each cell line used

Exceptionally similar trends between HTS studies and shake flask

Validation of HTS Approach

Peak productivity levels and VCD – HTS plate and Shake Flask Comparison



Peak productivity levels and VCD, are graphed as percent of total in order to reveal overall trends between shake flask and plate studies done with CHO A, B, C and D cell lines. Trends are remarkably similar validating the use of deep-well plates for large-scale screening.

Component Concentration Study

- Summarized ANOVA results for the component concentration study
- Significant differences were detected for CHO B and D cell lines in both peak viable cell densities (VCD) and productivity, where starred (*) p-values are less than 0.05

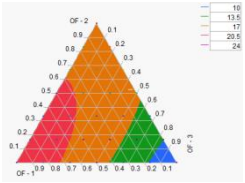
Cell Line	Data Analyzed	ANOVA Results
CHO A	Peak VCD	F(9,70) = 1.32, p = 0.242
CHO B	Peak VCD	F(9,70) = 2.90, p = 0.006*
	Peak Productivity	F(9,70) = 13.75, p = 0.0001*
CHO D	Peak VCD	F(9,70) = 2.87, p = 0.0061*
	Peak Productivity	F(9,70) = 5.20, p = 0.0001*
CHO E	Peak VCD	F(9,70) = 1.83, p = 0.077

Identification of Universal Blend

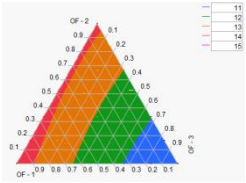
Leverage Metabolic Pathway Design Process

- Primary screening
  - Four different CHO cell lines
  - 96-deep-well plates
  - Shake flask studies
- Further optimization
  - Shake-scale DoE design
- Final formulation confirm in shake
- Verification at scale in bioreactor

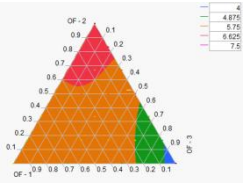
Ternary plots to select optimal formulation



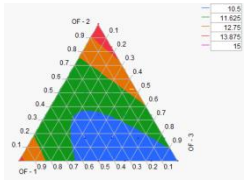
CHO A, Peak VCD



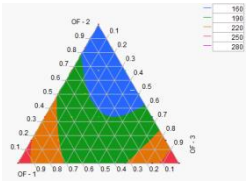
CHO B, Peak VCD



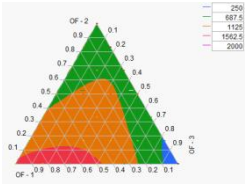
CHO D, Peak VCD



CHO E, Peak VCD



CHO B, Peak IgG Productivity



CHO D, Peak IgG Productivity

Ternary plots reveal hot spots for promising mixtures using optimal formulations one, two and three. Red and orange areas are most favorable. Mixtures were tested on several cell lines in an attempt to find the most universal blend.

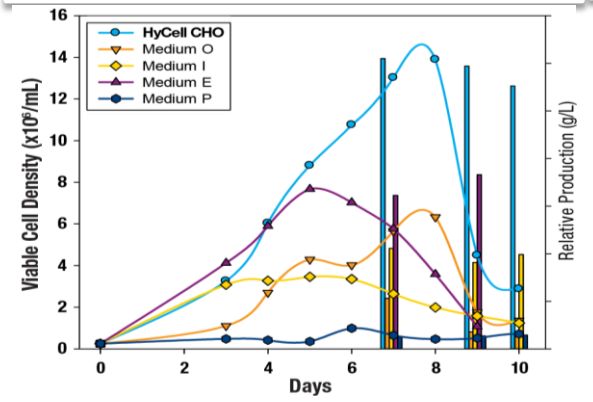
Versatile performance across variety of CHO clones

Thermo Scientific HyCell CHO Medium Performance Comparison

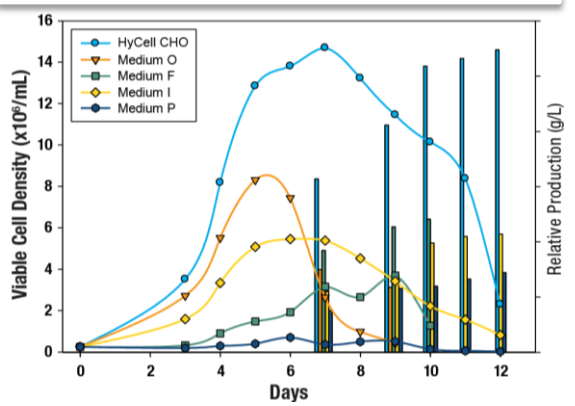
Universality of performance across various CHO lines

- Final formulation was evaluated in shake flasks
- IgG: biolayer interferometry
- Increased viability, cell densities and productivities

CHO-DG44 Cell Line



CHO-S Cell Line



Cultures were straight batch culture format, seeded at 0.25 x 10^6 viable cells/mL into 35mL volume in 125mL shake flasks, incubated at 37C, 5% CO_2. Cell counts and viabilities determined with a Beckman Coulter Vi-Cell™ automated cell counter (Trypan blue exclusion technique). Protein of interest quantitated via specific ELISA.

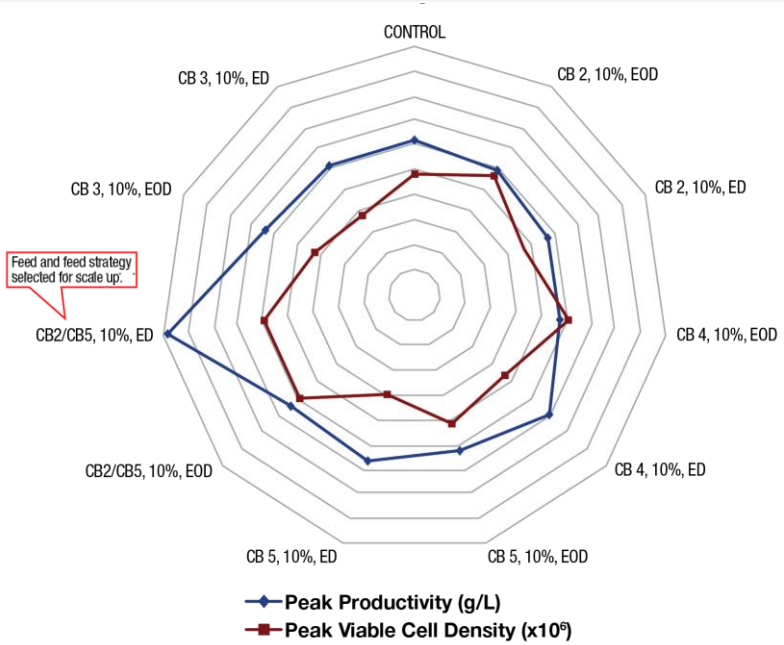
Superior productivity and VCD

Feed Optimization for Fed Batch Process

Leverage Metabolic Pathway Design Process

- Feed formulations and strategies
- Evaluating feeds at numerous concentrations ranging from 2.5% v/v to as high as 10% v/v.
- Feed schedule
- Final formulation was evaluated at shake flask level
- Verification in large-scale bioreactor runs

CHO Clone B in HyCell CHO Medium



CHO Clone B peak productivity (µg/mL) and peak viable cell density (x10^6 cells/mL) of various feed and feed strategies grown in HyCell CHO medium. Top 10 conditions in study represented. Data points furthest from center are higher in value

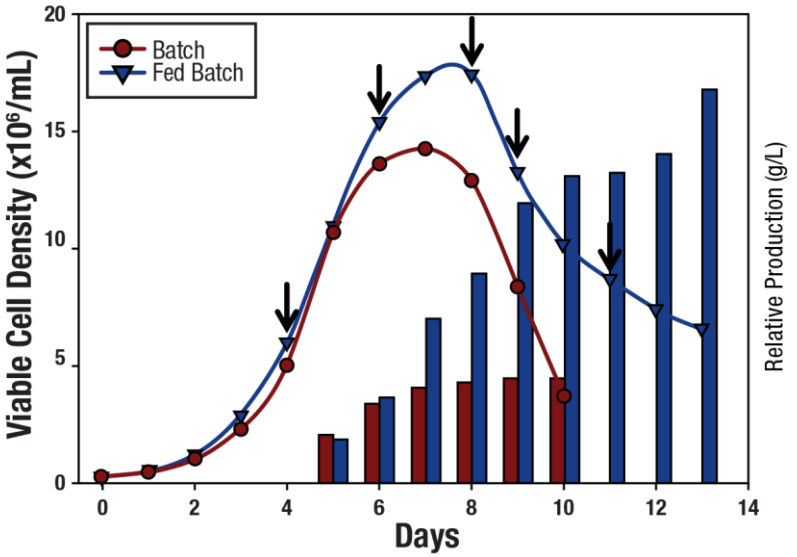
Cell Boost combination gives optimized fed batch process

Thermo Scientific HyCell CHO Medium Performance in Fed Batch

Verification in large-scale bioreactor runs

- Verification in large-scale bioreactor runs
- Feeds: mixture of Cell Boost 2 and Cell Boost 5 at 6:4 ratio
- Feeds of 6% (w/v) concentration delivered at 10% (v/v) of cell culture at times indicated by arrows

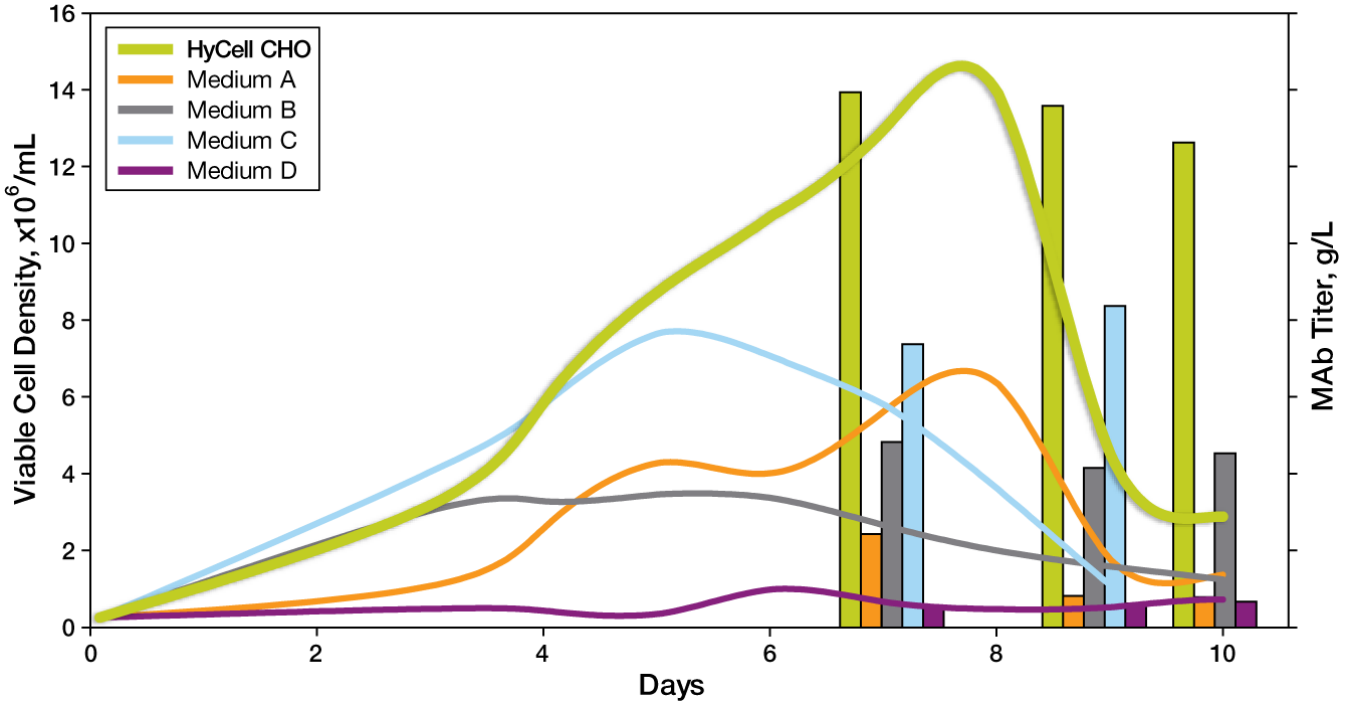
CHO-K1 Cell Line



Growth and productivity of a CHO-K1 derived cell line in HyCell CHO medium, comparing batch to fed-batch culture modes in a 10L bioreactor. Optimized feed was delivered at times indicated. Cell counts and viabilities determined with a Beckman Coulter Vi-Cell automated cell counter (Trypan blue exclusion technique). [IgG] determined via biolayer interferometry on a forteBIO Octet QK 384 system (protein A based probes).

Cell Boost combination and feeding strategy enhances yields

Thermo Scientific HyCell CHO Medium



- Animal-derived component-free
- Chemically defined
- Performance across broad variety of clones
- Dramatically outperforms next closest commercially available media by nearly five-fold
- Supports growth to high cell density and high productivity with comparative higher yields
- Delivers significant cost savings

Why Choose Thermo Scientific Media?

High Quality Standards

- ISO 9001 (2008)
- ISO 13485 (2003)
- EudraLex Annex 1
- Medical Device cGMP 21 CFR820

Robust Service Offering

- Media and feed formulation
- Media optimization and process development
- Rapid response production

Optimized for your Process

- Animal-derived component free
- Serum-free media
- Protein-free media
- Chemically-defined media

Your Partner In Science

ThermoFisher
SCIENTIFIC

The world leader in serving science

Committed to your goals
for better results and greater productivity