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TUTORIAL

Improving Medium Transitions

Techique Makes It Possible to Use One Medium from Development to Manufacturing

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The high specificity that is characteristic of a humanized monoclonal antibody makes it an ideal tool for cancer therapies. The ability to successfully reduce the uncertainties around development and to augment the efficacy of the final product is very much dependent on establishing a process that allows reproducibility from development to manufacturing.

During development of an antibody biologic as an anticancer medicine, various activities, such as clone selection, expansion, and verification, often require different medium requirements, calling for multiple media. Transitioning from one medium to another typically poses a certain level of risk in maintaining the clone of interest.

Irvine Scientific developed BalanCD™ CHO Growth A as a chemically defined, high-performance CHO production medium for batch and fed-batch processes. Experiments were designed to evaluate the potential of BalanCD CHO Growth A for both supporting early cell-line development activities and for determining its scalability to larger-scale production.

BalanCD CHO Growth A was evaluated, together with 10% FBS containing DMEM/F12 and one other commercially available CHO cloning medium (Media S), for single-cell subcloning in a limiting dilution assay. Using three monoclonal antibody-expressing CHO lines (CHO-M, CHO-S, and CHO DXB11), cells were seeded in 96-well plates at a calculated 1 cell/well in each test medium.

Plates, incubated at 37°C, 5% CO_2 in a humidified incubator, were evaluated on day 14 using light microscopy and scored for colony growth (*Figure 1*). Cells plated in BalanCD CHO Growth A formed more colonies than the 10% FBS condition for all three cell lines. The average colony size in the BalanCD CHO Growth A condition was comparable to that in the 10% FBS condition (image not shown) and larger than that in the Media S cloning medium. BalanCD CHO Growth A appears to be effective in the single-cell cloning applications.

CHO-S clones isolated from the limiting dilution assay were used, and high-producing clones were selected for scaleup from 96-well plates to 6-well plates, to 50 mL bioreactor tubes, and finally to 125 mL shake flasks. Once in shake flasks, the resulting clones were evaluated in fed-batch mode using BalanCD CHO Growth A and fed with BalanCD CHO Feed 1.

Cultures, which were fed seven times 5% of the initial working volume on days 2, 4, 6, 8, 10, 12, and 15, were maintained at 37°C, 5% CO_2 in a humidified incubator and agitated at 120 rpm on an orbital shaker. Cultures were inoculated at 2×10^5 cells/mL.

Cell counts were determined using a Vi-Cell™ XR cell viability analyzer (**Beckman Coulter**). Glucose (6 g/L)

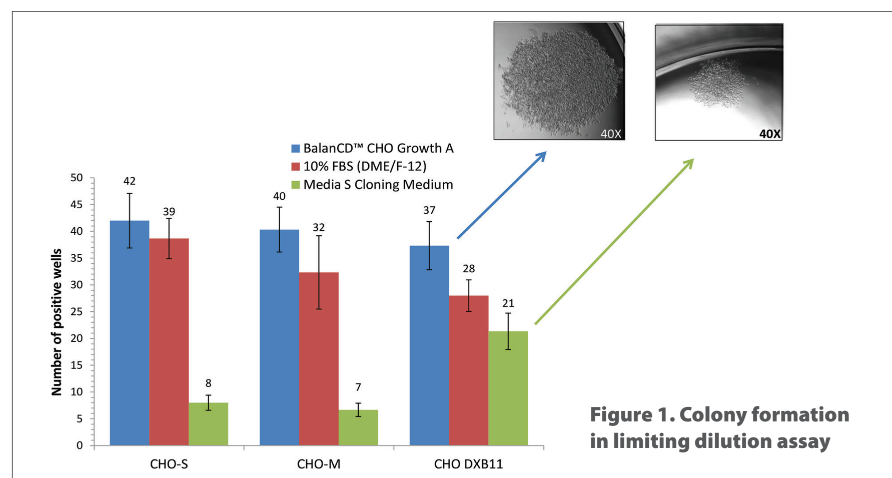

Figure 1. Colony formation in limiting dilution assay

was fed to all cultures when glucose fell below 2 g/L. Glucose levels were monitored using a BioProfile® FLEX (**Nova Biomedical**). Antibody titer was quantified using an octet® QKe from **fortéBIO**.

Media S cloning medium failed in scale-up as it did not support normal-density cultures. The serum-containing medium was not tested because its use as an expansion medium would not be suitable.

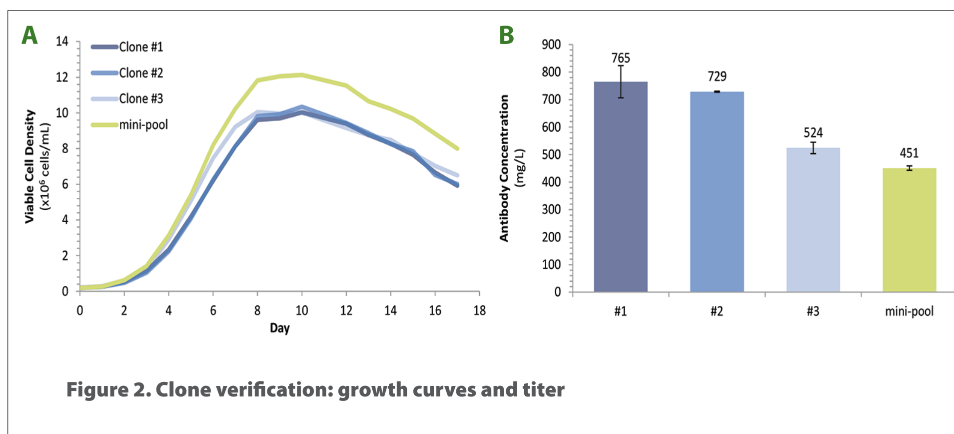

Figure 2. Clone verification: growth curves and titer

Performance of the top three clones from the BalanCD CHO Growth A subcloning group was evaluated relative to the original CHO-S mini-pool, from which all subsequent CHO-S clones were derived, in fed-batch using BalanCD CHO Growth A and BalanCD CHO Feed 1 (*Figure 2*).

Although growth was slightly reduced relative to the mini-pool (*Figure 2A*), the highest-producing clone (#1) achieved a 70% improvement in titer over the mini-pool (*Figure 2B*), demonstrating successful clone selection and expansion in BalanCD CHO Growth A.

Automated Single-Cell Cloning in a Semi-Solid Medium

BalanCD CHO Growth A was also evaluated for single-cell subcloning using a ClonePix™ FL imager from **Life Technologies** (*Figure 3*). Semi-solid media was prepared by mixing equal parts of liquid concentrate (2×) BalanCD CHO Growth A and CloneMatrix© (**Molecular Devices**) and supplementing with 6 mM L-glutamine and CloneDetect™ FITC. Cell density and viability of a two-day-old CHO-M culture was determined using a Guava® (**EMD Millipore**) cell counter.

The medium was inoculated at 200 cells/mL. The cell suspension was plated into two six-well plates with 2.5 mL/well. Plates were incubated at 37°C, 5% CO_2, in a humidified incubator. ClonePix images were evaluated on days 10 and 15 to determine colony growth. High-secreting colonies were picked and transferred to 96-well plates. Antibody titer was quantified on day 7 by ELISA.

When evaluated on day 10 and day 15, 1193 and 1585 colonies were observed, respectively. The ranking plots (*Figures 3A & 3B*) depict the resulting colony size distribution. The inset plots depict the size distribution of the 100 largest colonies.

The ranking plot of antibody concentration (*Figure 3C*) depicts the titer distribution of the 42 highest-producing clones. This evaluation demonstrates that BalanCD CHO Growth A is effective for automated single-cell cloning in a semi-solid medium.

This study demonstrates the utility of BalanCD CHO Growth A medium as a cloning medium and proves its scalability. Its compatibility and performance in supporting clonal cell selection and expansion has also been shown.

The ability to use a single, chemically defined medium mitigates the risk involved with switching media throughout development. GEN

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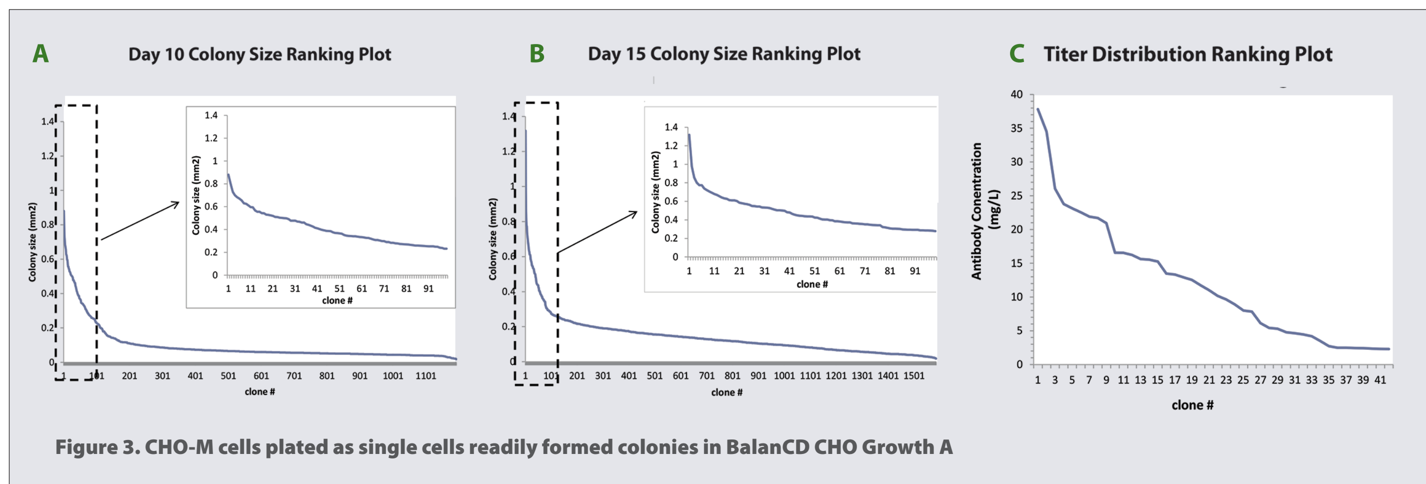

Figure 3. CHO-M cells plated as single cells readily formed colonies in BalanCD CHO Growth A