

Enhancing Clonal Outgrowth: Optimal Strategies with InstiGRO® CHO PLUS

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Introduction

The demand for protein therapeutics has soared since their introduction, driving the need for improved production methods which ensure both higher capacity and consistent product quality. Chinese Hamster Ovary (CHO) cell lines are often the mammalian cell line of choice due to their rapid growth, and ability to express foreign proteins, which result in high product yields. Unfortunately, the development of stable cell lines for manufacturing of protein therapeutics can be a significant challenge due to the numerous cell culture bottlenecks. Should the metabolic needs of the cells not be met following single cell isolation, clonal outgrowth can be very low. This amounts to increased project expenditure and timelines for both Contract Development Manufacture Organisations (CDMOs), such as UGA Biopharma, and independent pharmaceutical companies.

Here we demonstrate that Advanced Instruments' (AI) proprietary InstiGRO™ CHO PLUS cell culture supplement is a proven solution for overcoming cell line development inefficiencies due to poor clonal outgrowth. InstiGRO™ CHO PLUS is an animal component free supplement that

was launched in 2019 to nurture single cells into healthy clonal colonies and improve the cloning efficiency of cell line development projects. Since the initial launch of the product, the supplements' formulation has been further optimized to enhance cloning efficiencies and average colony size. This study aims to show how the new InstiGRO CHO PLUS, in combination with UGA Biopharma's First CHOice® medium, can further expedite stable cell line development compared to the original InstiGRO CHO PLUS formulation. Two CHO cell lines, both of which were expressing monoclonal antibodies (mAbs), were single cell seeded using both the new and original formulation of InstiGRO CHO PLUS. The average percentage confluence of clones and average cloning efficiency were used to determine the success of the new InstiGRO CHO PLUS. We find that the average percentage clonal outgrowth increased as much as 16.7% when the new formulation of InstiGRO CHO PLUS was applied compared to the original. Similarly, the average percentage confluence of clones on day 14 was improved by up to 24.1%.

Materials and Methods

Cells, Medium and Supplements

Pool 1 and 2, both of which were expressing mAbs, were used to evaluate the new and original formulation of InstiGRO CHO PLUS. A control supplement was also included in the study to provide a benchmark for the performance of the InstiGRO CHO PLUS formulations. In terms of cell culture medium, the supplements were partnered with UGA Biopharma's First CHOice medium versus an alternate commercially available medium, 'Medium A'.



Single-cell seeding via limiting dilution

Single cells were deposited into 96-well plates (CytoOne® Plate, Starlab, CC7682-7596) via limiting dilution (0.75 cells/well). A total of 12x 96 well plates were seeded per pool. The conditions are summarised in Table 1.

Condition	Number of Plates
New InstiGRO CHO PLUS with First CHOice Medium w.o. HT	3
Original InstiGRO CHO PLUS with First CHOice Medium w.o. HT	3
Control supplement with First CHOice First CHOice Medium w.o. HT	3
New InstiGRO CHO PLUS with Medium A	1
Original InstiGRO CHO PLUS with Medium A	1

Table 1. List the conditions for each of the 12 plates set up per pool.

Clonal outgrowth and confluence assessment with Cell Metric®

Following seeding, plates were incubated for 14 days at 37°C and 8% CO₂. Whole well images were captured using Advanced Instruments' Cell Metric®. On day 14, clonal outgrowth analysis was performed using the Cell Metric software, and the percentage clonal outgrowth was calculated from the total number of single seeded wells. Note that only colonies with confluence >5% on day 14 were considered in the calculations.

$$\text{Percentage clonal outgrowth} = \frac{\text{Number of clonal colonies}}{\text{Number of single cell seeded wells}} \times 100$$

Once the percentage clonal outgrowth for each plate had been calculated, the averages and standard deviations across the plates were calculated, when appropriate.

The Cell Metric's confluence overlay was used to assess the percentage confluence of colonies on day 4, 7, 11 and 14. Once the confluence percentage had been noted for each timepoint, the averages and standard deviations across the plates were calculated, when appropriate.



Statistical analysis

Statistical analysis was carried out using GraphPad Prism. An unpaired t-test was used to determine significance between the average cloning efficiency and average colony size on day 14 following single cell seeding with First CHOice Medium w.o. HT in combination with the new InstiGRO CHO PLUS versus the original formulation ($p < 0.05$ = significance; $p > 0.05$ = not significant). Where no statistical significance was found, an effect test was estimated using Cohen's *d*, see Table 2.

Cohen's <i>d</i>	Effect Size
0.2	Small
0.5	Medium
0.8	Large

Table 2. Shows the Cohen's *d* values used to determine the effect in this study.



Results

Significant improvement in clonal outgrowth

The average percentage clonal outgrowth for Pool 1 was highest in plates seeded with the new InstiGRO CHO PLUS formulation and UGA Biopharma's First CHOice medium. On average, 25.5% of single seeded wells gave rise to colonies with confluence >5% on day 14 (n = 3). When First CHOice medium was supplemented with the original InstiGRO CHO PLUS, the average clonal outgrowth was 14.8%, by comparison (n = 3). While no statistical significance was determined ($p = 0.12$), the effects test showed that the new InstiGRO CHO PLUS had a large effect on the cloning efficiency, compared to the original formulation ($d = 1.66$) (see Figure 1).

Similar trends can be seen in results from the cloning experiment with Pool 2. The average percentage clonal outgrowth was highest when the new InstiGRO CHO PLUS was used in combination with First CHOice medium. On average, 31.0% of single seeded wells resulted in colonies with confluence >5% on day 14 (n = 3). When First CHOice medium was supplemented with the original InstiGRO CHO PLUS, the average clonal outgrowth was 14.3%, by comparison (n = 3). This effect was characterised as large ($d = 2.61$), despite no statistical significance being found ($p = 0.06$) (see Figure 2).

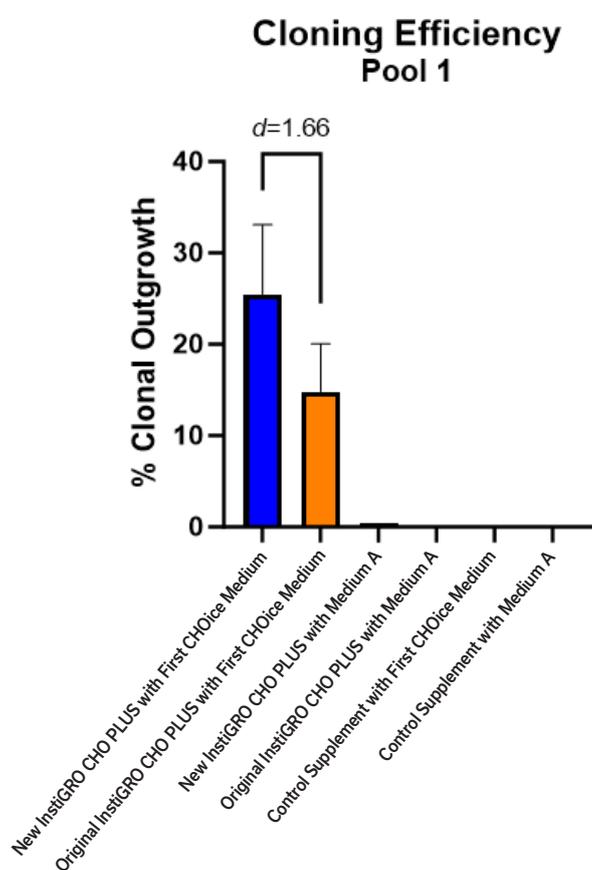


Figure 1. Shows the average percentage clonal outgrowth of Pool 1 following limiting dilution with the various cell culture supplements and cell culture mediums. Error bars show the standard deviation between replicates (n = 3). 'd' indicates Cohen's d which was used to characterise the size of the effect.

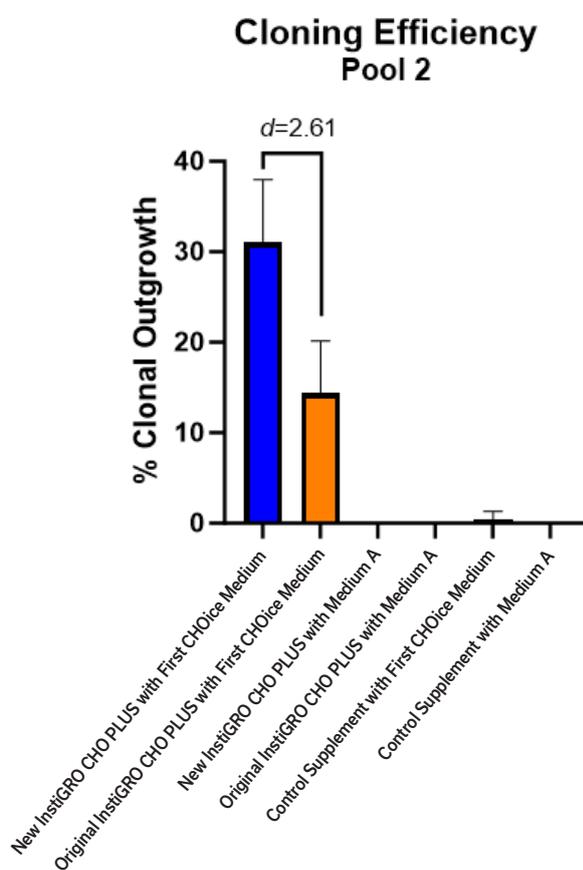


Figure 2. Shows the average percentage clonal outgrowth of Pool 2 following limiting dilution with the various cell culture supplements and cell culture mediums. Error bars show the standard deviation between replicates (n = 3). 'd' indicates Cohen's d which was used to characterise the size of the effect.

Enhanced confluence for clonal colonies

The average percentage confluence of Pool 1's clonal colonies was highest when the new InstiGRO CHO PLUS was used in combination with First CHOice medium. On day 14, the average confluence of colonies was 30.9% (n = 3). By contrast the average confluence of clones on day 14 was 18.3% when the original InstiGRO CHO PLUS was used with First CHOice medium. Finally, the average confluence was 6.9% when the new InstiGRO CHO PLUS and Medium A were applied (n = 1). While no statistical significance was determined in the application of the new versus original InstiGRO CHO PLUS in combination with First CHOice medium (p = 0.07), the effects test showed that the new InstiGRO CHO PLUS had a large effect on the average colony size, compared to the original formulation (d = 1.95) (see Figure 3).

Results from Pool 2's cloning experiment were comparable – The average percentage confluence was highest in plates seeded with the new InstiGRO CHO PLUS and First CHOice medium (49.0% on day 14, n = 3). Average percentage confluence was 24.9% when the original InstiGRO CHO PLUS formulation was used in combination with First CHOice medium, by comparison. This effect was characterised as large (d = 3.66) and statistically significant (p = 0.03) (see Figure 4).

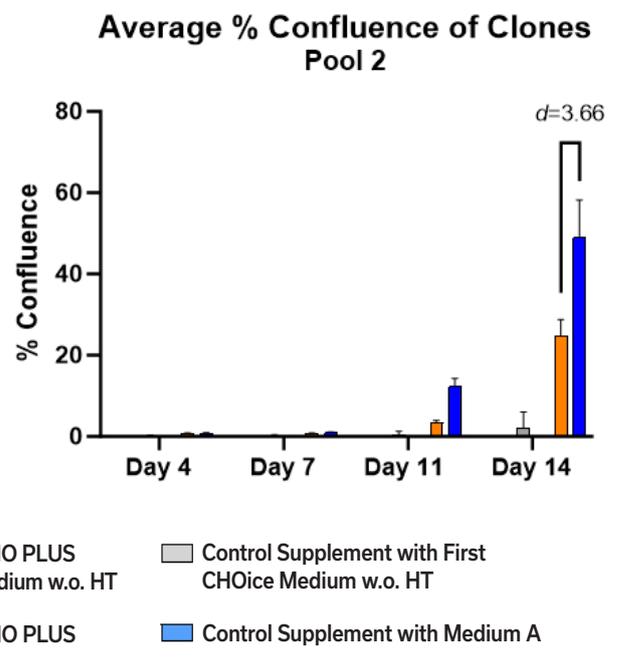
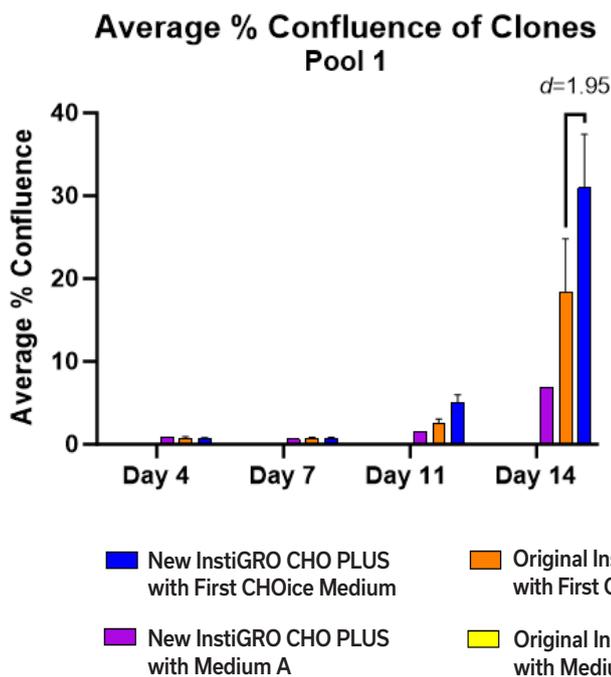


Figure 3. Shows the average percentage confluence of Pool 1 clones on day 4, 7, 11 and 14 following single cell seeding. Error bars show the standard deviation between the average percentage confluence per plate (n = 3). 'd' indicates Cohen's d which was used to characterise the size of the effect.

Figure 4. Shows the average percentage confluence of Pool 2 clones on day 4, 7, 11 and 14 following single cell seeding. Error bars show the standard deviation between the average percentage confluence per plate (n = 3). 'd' indicates Cohen's d which was used to characterise the size of the effect.

Discussion and Conclusions

To conclude, the new InstiGRO CHO PLUS formulation significantly improved both the average cloning efficiency and average colony size of the two cell lines included in the study. While InstiGRO CHO PLUS was originally launched in 2019 to address the metabolic needs of cells following single cell isolation, further optimisation has extended the product's potential to nurture cells into healthy clonal colonies.

The average percentage clonal outgrowth was increased by up to 16.7% when the new formulation of InstiGRO CHO PLUS was used to supplement UGA's First CHOice medium. Similarly, the average percentage confluence of clones on day 14 was increased by up to 24.1%. In terms of benefits, the new InstiGRO CHO PLUS not only enables CDMOs and independent pharmaceutical companies to reduce the number of plates they seed per project, but also allows clonal expansion to commence at an earlier timepoint. Put together, project costs and timelines can be significantly reduced, allowing groups to meet the rising demands for new protein therapeutics.

Introducing UGA Biopharma

UGA Biopharma was established in 2009 in Hennigsdorf near Berlin, Germany. The company's international team of experts specializes in the development of monoclonal cell lines for the manufacture of biologics and biosimilars. Alongside UGA offers ready-to-use (R2U) cell lines for the expression of biosimilar candidates, customized First CHOice® feed and medium platform for cell cultures which provides you with a wide range of options.



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