An Easy-to-Use Media Supplement for Increased Biomolecule Galactosylation

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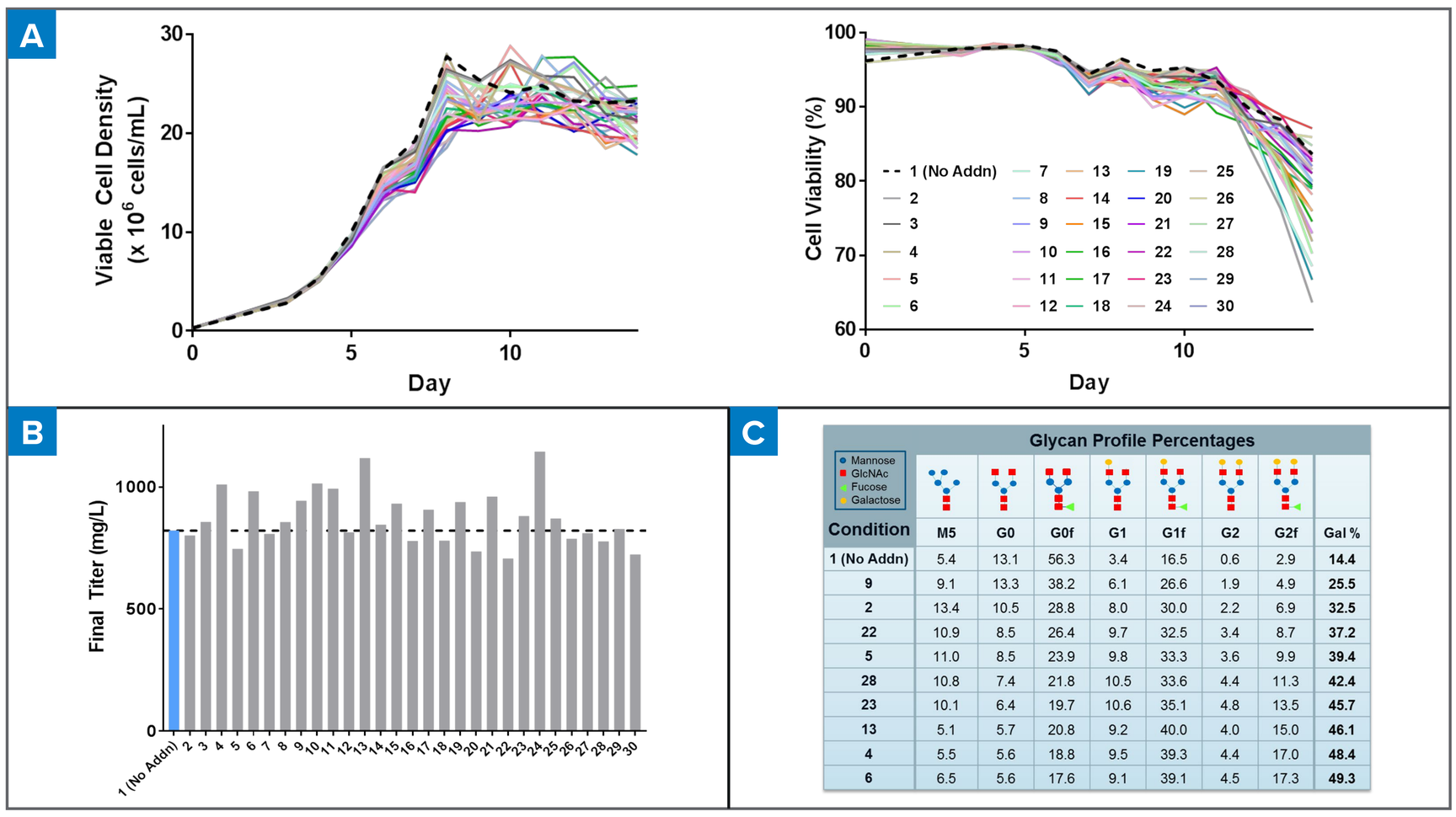
Introduction

The production of therapeutic antibodies (Abs) requires high product titers and excellent product quality to ensure efficient manufacturing and potent drug efficacy. Glycosylation, or the attachment of sugars to organic molecules, is a critical quality aspect that can significantly alter Ab binding, function, and therapeutic effect. Galactose is a key sugar of interest due to its significant impact on Ab function and the ability to control galactosylation through cell culture medium.

FUJIFILM Irvine Scientific's approach of Rational Culture Media Design was applied to the development of a supplement that would increase biomolecule galactosylation. Various components were assessed for their ability to modulate levels of galactose on a model therapeutic Ab produced in Chinese hamster ovary (CHO) fed-batch cultures. BalanCD Gal Supplement was formulated and compared to galactose-modulating supplements from other suppliers. BalanCD Gal Supplement and one supplement from another supplier showed minimal impact to cell growth and titer while significantly increasing galactosylation. Furthermore, an *in vitro* complement-dependent cytotoxicity (CDC) assay was utilized to evaluate Ab efficacy. Even at half the concentration of the other supplier's supplement, BalanCD Gal Supplement resulted in the highest percent galactosylation and Ab function.

Overall, BalanCD Gal Supplement increased Ab galactosylation and consequently Ab function. BalanCD Gal Supplement outperformed all other suppliers' supplements and resulted in the best overall cell growth, glycan profile, and functional Ab activity. In addition, it is easier and simpler to use with a one-day bolus addition. The ability to titrate BalanCD Gal Supplement, measure glycosylation levels, and evaluate antibody function provides cohesive control of antibody product quality.

Results



Condition	M5	G0	G0f	G1	G1f	G2	G2f	Gal %
1 (No Addn)	5.4	13.1	56.3	3.4	16.5	0.6	2.9	**14.4**
9	9.1	13.3	38.2	6.1	26.6	1.9	4.9	**25.5**
2	13.4	10.5	28.8	8.0	30.0	2.2	6.9	**32.5**
22	10.9	8.5	26.4	9.7	32.5	3.4	8.7	**37.2**
5	11.0	8.5	23.9	9.8	33.3	3.6	9.9	**39.4**
28	10.8	7.4	21.8	10.5	33.6	4.4	11.3	**42.4**
23	10.1	6.4	19.7	10.6	35.1	4.8	13.5	**45.7**
13	5.1	5.7	20.8	9.2	40.0	4.0	15.0	**46.1**
4	5.5	5.6	18.8	9.5	39.3	4.4	17.0	**48.4**
6	6.5	5.6	17.6	9.1	39.1	4.5	17.3	**49.3**

FIGURE 1. One of the design of experiment (DoE) studies to develop BalanCD Gal Supplement for increased biomolecule galactosylation. Various supplement conditions were added to CHO fed-batch cultures expressing an IgG1 Ab and analyzed for impact on cell growth, Ab production, and product quality.
(A) Viable cell density and cell viability of the 30 DoE fed-batch conditions. Galactose-modulating supplements did not significantly impact cell growth as viable cell densities and cell viabilities were similar to the control (Condition 1: No Addn).
(B) Final Ab titer of the 30 DoE fed-batch conditions. Galactose-modulating supplements had a limited effect on Ab titer levels compared to the control (Condition 1: No Addn; blue bar).
(C) Percentage breakdown of the glycan profiles from a set of the DoE fed-batch conditions. M5 levels were below 15% and the G0 glycan percentages decreased as G1 and G2 percentages increased. Percent galactose, defined as the number of galactoses divided by the number of possible galactoses, ranged from a low of 14% to a high of 45%.

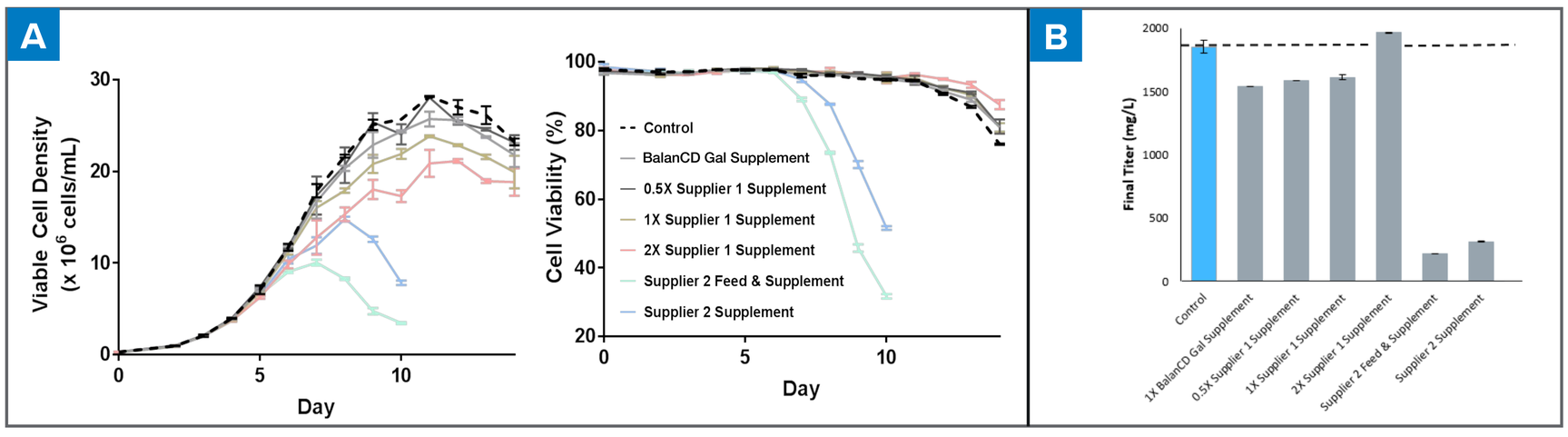


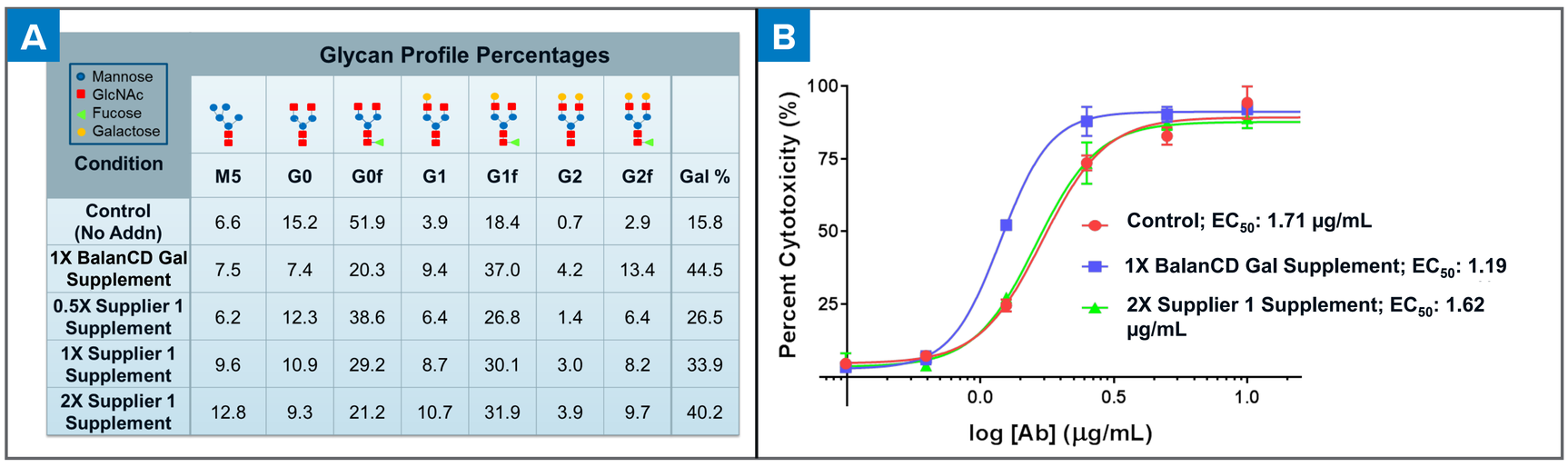
FIGURE 2. Verification of the BalanCD Gal Supplement and comparison to supplements from other suppliers. Supplements were added to CHO fed-batch cultures according to manufacturer's instructions and analyzed for impact on cell growth and Ab production. **(A)** Viable cell density and cell viability of the cultures with and without galactose-modulating supplements. BalanCD Gal Supplement and Supplier 1 Supplement did not significantly impact cell growth as viable cell density and cell viability were similar to the control. Supplier 2 Supplement significantly lowered viable cell density and cell viability; the cultures ended prematurely since viability dropped below 70%. **(B)** Final Ab titer of the cultures with and without galactose-modulating supplement. BalanCD Gal Supplement and Supplier 1 Supplement had a limited impact on Ab titer while the Supplier 2 Supplement significantly decreased titers due to stunted cell growth and viability. Due to poor growth and titer levels, Supplier 2 was excluded from further studies.

Methods

Fed-Batch Cultures
- CHO cells expressing IgG1 Ab against CD20
- Culture vessel: 125 mL shake flasks
- Seeding density: 0.3 x 10^6 cells/mL
- BalanCD CHO Growth A and 4% BalanCD CHO Feed 4 on culture days 3-7
- Other supplier feeds and supplements were used as directed

Culture Analysis
- Viable cell density and cell viability: Beckman Vi-Cell XR
- Ab titer: Pall FortéBio QK^e
- Glycan analysis: PerkinElmer LabChip GXII

CDC Assay
- Daudi B lymphoblast cells
- Normal human complement serum
- Promega CytoTox-Glo® cytotoxicity assay



Condition	M5	G0	G0f	G1	G1f	G2	G2f	Gal %
Control (No Addn)	6.6	15.2	51.9	3.9	18.4	0.7	2.9	15.8
1X BalanCD Gal Supplement	7.5	7.4	20.3	9.4	37.0	4.2	13.4	44.5
0.5X Supplier 1 Supplement	6.2	12.3	38.6	6.4	26.8	1.4	6.4	26.5
1X Supplier 1 Supplement	9.6	10.9	29.2	8.7	30.1	3.0	8.2	33.9
2X Supplier 1 Supplement	12.8	9.3	21.2	10.7	31.9	3.9	9.7	40.2

FIGURE 3. Glycan and CDC analysis of Abs. (A) Abs from cultures with high viable cell density, viability, and titers were further analyzed for their glycan profiles. BalanCD Gal Supplement and 2X Supplier 1 Supplement resulted in Abs with galactose percentage levels of about 45%. The lower concentrations of Supplier 1's Supplement resulted in lower galactose percentages and were excluded from further studies. **(B)** Abs that passed the requirements of high viable cell density, viability, titer, and galactosylation were evaluated for their therapeutic effect and ability to induce *in vitro* CDC. Increased galactosylation by BalanCD Gal Supplement resulted in more therapeutic Abs with lower EC_{50} values than the control. Conversely, the high galactosylation from the 2X Supplier 1 Supplement did not result in as low of an EC_{50} value compared to the Abs from the BalanCD Gal Supplement.

Summary

Comparison of key features and performance of BalanCD Gal Supplement to alternatives

	Supplement Addition Strategy	Max VCD (x 10^6 cells/mL)	Estimated CCD (x 10^6 cells/mL)	Final Titer (mg/L)	Percent Galactosylation (%)	Man 5 (%)	CDC EC_{50} (µg/mL)
Control, No Supplements	N/A	28.2	198	1850	15.8	6.6	1.71
1X BalanCD Gal Supplement	Bolus addition	25.7	185	1538	44.5	7.5	1.19
2X Supplier 1 Supplement	5 every other day additions	21.1	148	1964	40.2	12.8	1.62