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ARTICLE

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Generation of reference cell lines, media, and a process platform for CHO cell biomanufacturing

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Abstract

Due to the favorable attributes of Chinese hamster ovary (CHO) cells for therapeutic proteins and antibodies biomanufacturing, companies generate proprietary cells with desirable phenotypes. One key attribute is the ability to stably express multi-gram per liter titers in chemically defined media. Cell, media, and feed diversity has limited community efforts to translate knowledge. Moreover, academic, and nonprofit researchers generally cannot study "industrially relevant" CHO cells due to limited public availability, and the time and knowledge required to generate such cells. To address these issues, a university-industrial consortium (Advanced Mammalian Biomanufacturing Innovation Center, AMBIC) has acquired two CHO "reference cell lines" from different lineages that express monoclonal antibodies. These reference cell lines have relevant production titers, key performance outcomes confirmed by multiple laboratories, and a detailed technology transfer protocol. In commercial media, titers over 2 g/L are reached. Fed-batch cultivation data from shake flask and scaled-down bioreactors is presented. Using productivity

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as the primary attribute, two academic sites aligned with tight reproducibility at each site. Further, a chemically defined media formulation was developed and evaluated in parallel to the commercial media. The goal of this work is to provide a universal, industrially relevant CHO culture platform to accelerate biomanufacturing innovation.

KEYWORDS
biopharmaceuticals, chemically defined media, monoclonal antibodies, platform process, reference CHO cell

1 | INTRODUCTION

Chinese hamster ovary (CHO) cell lines have been preferentially used to produce recombinant therapeutics and are used for manufacturing approximately 70% of all recombinant biopharmaceutical proteins and monoclonal antibodies (mAbs) approved since 2016 (Lu et al., 2020). A key step to developing such medicines is the development of stable and high-productivity cell lines, and the use of robust and reliable manufacturing processes. CHO cells have been commonly used in the industry because these cell lines are easy to transfect, have been adapted to perform well at commercial manufacturing scale, have rapid growth rates, and can perform human-compatible posttranslational modifications. The culmination of advances in cell line, media, and process development over the past 30 years has led to upstream processes with titers as high as 13 g/L in fed-batch production (Kelley, 2009; Stolfa et al., 2018). As many of these advances have commercial value for the originator companies, CHO cells, along with the media and process conditions are highly proprietary in nature. Companies continue to invest significant resources to develop host cells as part of their platform processes, in addition to custom media and feed blends necessary to ensure robust, reliable, and productive platforms. Meanwhile, academic, and nonprofit researchers often do not have access to high-producing cell lines, proprietary knowledge, or advanced automation resources, to generate cells with similar performance characteristics. As a result, the scientific discoveries made by these researchers using relatively low-producing cells (e.g., <1 g/L) may not translate well to industrially relevant cell lines. Another consideration is that students working in an academic environment do not gain critical experience working with cells capable of growing at high cell densities and with high productivities, which impacts workforce development for the sector.

Through an industry-university cooperative research center (IUCRC), funded by the U.S. National Science Foundation, known as the Advanced Mammalian Biomanufacturing Innovation Center (AMBIC), comprised of five universities and more than 25 companies, we developed an upstream process for the growth and production of antibodies using two reference cell lines on chemically defined media. The production characteristics of these cells are described with the intent that these cells would be available to the broad research community for noncommercial use to advance the field and the specification of a chemically defined media is intended to catalyze broad-based understanding of medium development activities for the community. We demonstrated repeatable growth and production using these cells in shake flasks and scale-down bioreactor systems at two different academic laboratories to ensure that the process technology can be transferred effectively. This work seeks to harmonize the field and provide standard datasets for comparison across academic and industry research sites for CHO cell biomanufacturing.

2 | MATERIALS AND METHODS

2.1 | Cell lines and commercial media

To address the need for CHO reference cell lines that demonstrate industrially relevant growth and productivity, two mAb production cell lines were used in this study. A CHO K1-derived cell line expressing the VRC01 monoclonal antibody (Clone A11) was generously donated from the Vaccine Production Program Laboratory at the U.S. National Institute of Health and will be noted as "VRC01" throughout the text. A second CHO GS knockout cell line (developed using the CHOZN platform), noted as GS23, was shared by MilliporeSigma (MilliporeSigma, 2020; SAFC, n.d.). Commercial media used in this work was ActiPro paired with the Cell Boost 7a/7b feed (Cytiva, Catalog no. SH31039.02, SH31026.07, and SH31027.01) for the VRC01 cell line and EX-CELL Advanced CHO Fed-batch Media with CHO Feed 1 (MilliporeSigma, Catalog no. 14366C, 24368C) for the GS23 cell line. Glutamine was supplemented at 6 mM for all VRC01 cultures. Data from these studies were generated at Clemson University and the University of Delaware. Product information for all supplies and materials used in this work is listed in Table S1.

2.2 | Media development and adaptation

To address the need for universal CHO reference media that is animal origin free, a new chemically defined media formulation

was generated as part of this work and other ongoing projects within AMBIC. Companies collaborated to generate a set of unoptimized media and feed formulations that were generally representative of those used by the industry, which is termed "AMBIC 1.0" (for media and feed). For relevant studies, cells were directly adapted to AMBIC 1.0 formulations until high cell viability (greater than 95%) was achieved. Cells were initially seeded at 0.5 million cells per mL in AMBIC 1.0 media and passaged every 3–4 days for 30 days until viability greater than 95% was observed for 3 consecutive passages. The formulation for AMBIC 1.0 media and feeds is provided in the Supplementary Information.

2.3 | Culture conditions in shake flasks

As an initial process validation, fed-batch shake flask studies were performed in 125-ml flasks with a working volume of 30 ml. The feeding schedule is shown in Table 1. At Site A, the glucose concentration was determined daily, and a bolus glucose feed was added to reach the target after the addition based on the target values listed in Table 1. At Site B, glucose bolus was provided daily or every other day in alignment with the feed schedule (100 µl for VRC01, 150 µl for GS23). The bolus glucose feed amounts were set based on previous experience with these cell lines and represent a similar total glucose volume between Site A and B. Samples were collected daily (after Day 3) for viable cell density, titer, and glucose/lactose assessment. At Site A, a 5% CO_2 incubator with an orbital shaker (VWR Advanced 3500) was used for shake flask cultures at 135 rotations per minute (rpm) with a 0.75-inch throw diameter. At Site B, a Multitron (InforsHT) shaking incubator that maintained 5% CO_2 and 80% relative humidity (RH) was used at 135 rpm with a 1-inch throw diameter.

2.4 | Culturing conditions in bioreactors

The ambr250 HT 12-way bioreactor system (Sartorius) was used for small-scale bioreactor studies at both sites. Key online parameters for the bioreactor cultures are shown in Table 1 (pH, temperature, dissolved oxygen (DO), seeding density, and feed concentrations). Before inoculation in the bioreactors, all cell lines were grown in shake flasks for a minimum of three passages after thaw to ensure viability exceeded 95% before bioreactor inoculation. For the VRC01 cell line, pH was set via a dead band to 6.9 ± 0.1 with 50% DO. The PID setting for the DO used a sequence of manipulated variables and PID values. These values are described in Harcum et al. (2022) as the third tuning final values (Harcum et al., 2022).

In ActiPro media, a previously optimized pyramid feed strategy was utilized (based on the suggestion of the cell line originator). Cell Boost 7a was provided at a feed ratio of 3% culture volume on Days 3–5, 4% on Days 6 and 7, and 5% feed ratio on Days 8 and 9. After this peak in feed content, the feed ratio stepped down to 4% on Day 10 and 11% and 3% on Days 12–14). Cell Boost 7b was provided daily at one-tenth the volume of Cell Boost 7a. For the VRC01 cell line grown in AMBIC 1.0 media, a single feed was provided at daily 3.0% at Site A and 3.3% at Site B.

For the GS23 cell line, pH was set to 7.2–7.4 for the first 24 h, followed by a shift down to 7.0–7.2 (as recommended by MilliporeSigma). Commercial media for the GS23 cell line (EX-CELL Advanced Fed-Batch) and AMBIC 1.0 media have a single component feed, provided at 5% every other day starting on Day 3. A concentrated glucose solution (45%) was added daily to achieve the desired setpoints shown in Table 1 and was calculated based on the daily measured glucose concentration for each bioreactor. Samples were collected daily for viable cell density counts, titer quantification, and metabolite monitoring.

TABLE 1 Feeding schemes and culture conditions for shake flask and bioreactors for two reference cell lines using two media types

Cell line	VRC01		GS23	
media	ActiPro with CellBoost 7a (7b)	AMBIC 1.0	EX-CELL Advanced CHO Fed-batch	AMBIC 1.0
Seed densities (x10^6 cells)	0.4	0.5	0.4	0.5
Feeding schedule	Pyramid feed strategy: daily: 3% (0.3%), 4% (0.4%), 5% (0.5%)	Daily: 3% (0.3%) (Site A) Daily: 3.3% (0.33%) (Site B)	5% every other day	5% every other day
Online pH setpoint	6.9		7.2–7.4 for 24 h, then 7.0–7.2	
Online dissolved oxygen (DO) setpoint (%)	50		30	
Glucose setpoint (g/L)	6.0, then 9.0 (after Day 5)	5.0	6.0	5.0

Note: Seed densities and feeding schedules were the same for shake flasks and bioreactors. The shake flask glucose additions are described in the Materials and Method section. For the bioreactors, pH and DO were controlled online.

2.5 | Analytical measurements

Viable cell density (VCD) and cell viability were quantified using a Vi-Cell XR Cell Viability Analyzer at both sites. Image analysis parameters for 50 acquired images were matched at both sites with the following specifications: 11.5-50-µm cell diameter, 85% cell brightness, 75% viable cell spot brightness, 5% viable spot area, and medium decluster degree. For mAb titer quantification from shake flasks, a Cedex Bio analyzer (Roche) was used at Site A and an Octet Red96e (Forte Bio) was used with Protein A biosensors at Site B. For bioreactor samples, the Octet was used for all titer quantification to align titers across sites. Antibody calibrators (Sartorius) were used to generate a standard curve for the Octet with cell culture supernatant samples diluted 10-fold with 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) and well mixed before analysis. For the Cedex Bio analyzer, a test kit for human IgG Bio was used (Roche) was used for mAb titer. The Cedex Bio analyzer was also used for metabolite quantification (glucose, lactate, glutamine, glutamate, and ammonia) with an auto-dilution feature for samples as needed at Site A. The reagent kits for these measurements are shown in Table S1. Osmolarity at Site A was determined using an OsmoTech PRO Multi-Sample Micro-osmometer (Advanced Instruments). Offline pH, pO_2, and pCO_2 were quantified with a blood-gas analyzer (BGA) ACL70 (Radiometer America). At Site B, metabolites (glucose, lactate, glutamine, glutamate, ammonia, sodium, calcium, and potassium), osmolality, and offline pH, pO_2, and pCO_2 were monitored using an at-line BioFlex2 with corresponding reagent cards and kits (Nova Biomedicals). All reagent cards and cartridges were installed according to manufacturer instructions with product numbers shown in Table S1. Samples were provided to BioFlex2 using the bioreactor liquid handling system with occasional manual sampling.

2.6 | Statistical analysis

All data are reported as mean ± standard error (SE). To determine statistical significance for cell-specific productivity between the sites, t-tests were used with a threshold of $p \leq 0.05$ and assumes the data is normally distributed. The web-based software tool Graphpad was used to perform the *t*-tests (https://www.graphpad.com/quickcalcs/ttest1/?format=SEM). The second measure of consistency between sites was the relative standard error (RSE), where an acceptable upper bound of 10% within each site and 20% between sites was established for maximum VCD, maximum titer, culture duration, and cell-specific productivity. These criteria were set based on extensive conversations with more than a dozen biopharmaceutical companies working collaboratively on this effort. While some companies preferred a narrower performance standard, others suggested more broad standards based on their own experiences. Ultimately, the group agreed that 20% variation between sites was acceptable as a reasonable industry-wide benchmark. These upper limits also align with the general acceptance of 10-15% variability for cell count data (Manahan et al., 2019) and are within the acceptable 30% RSE as outlined by the Center for Disease Control (Klein et al., 2002).

3 | RESULTS AND DISCUSSION

The VRC01 and GS23 cell lines were studied in both shake flask and scale-down bioreactors to assess the reproducibility of growth, metabolite, and production titer profiles within each site and across the two sites. The feeding schemes and other culture conditions including online bioreactor control setpoints are provided in Table 1. The overall goal was to demonstrate that these cell lines are robust and able to achieve high titers in commercial and consortium-designed media with consistent and reproducible performance.

3.1 | Growth characterization and mAb production in shake flask

3.1.1 | VRC01 performance in shake flasks

Growth characteristics for the shake flask fed-batch cultures for VCR01 at both sites using the commercially available media are shown in Figure 1 and Table 2. Viable cell density (VCD), titer, along with glucose and lactate concentration profiles for both cell lines as shown as duplicate cultures at each site in Figure 1a–d. The VRC01 cell line (Figure 1a–d) was cultured in ActiPro with 6 mM glutamine using the corresponding Cell Boost 7a and 7b feed system. Cultures were terminated when viability dropped below 70%. The observed growth profile shapes are remarkably similar between the sites despite the different absolute maximum VCDs. Cultures at both sites reached approximately 20 million cells/ml on Day 6. The VCD at Site A reached approximately 25 million cells/mL, while the maximum VCD at Site B reached 35 million cells/ml (Figure 1a). While the same shake speed (135 rpm) was used at both sites, the shaker throw diameter was 0.75 inches at Site A and 1 inch at Site B. This unequal throw diameter likely resulted in lower oxygenation at Site A and thus a lower maximum VCD as previously observed (Klaubert et al., 2021). In addition to the oxygenation, the pH (uncontrolled and not monitored) may vary between sites. Despite these differences, the glucose, lactate, and mAb titer profiles across sites (Figure 1b–d) were similar. The cultures at Site A reached a maximum titer of 2.25 g/L (Day 13), while the maximum titer at Site B was 1.94 g/L (Days 12–14), demonstrating high titers. The glucose profiles were similar across the sites with slightly lower concentrations observed at Site A due to the calculated target glucose approach versus a fixed volume, as described in Section 2.3. At Site B, the pre-determined daily bolus of 100 µl glucose was sufficient to maintain glucose levels between 2 and 6 g/L until Day 9 at Site B. Glucose was fully consumed in cultures at Site A, despite the lower observed VCD, likely attributable to less efficient glucose metabolism due to lower oxygen levels due the smaller throw diameter on the shaker

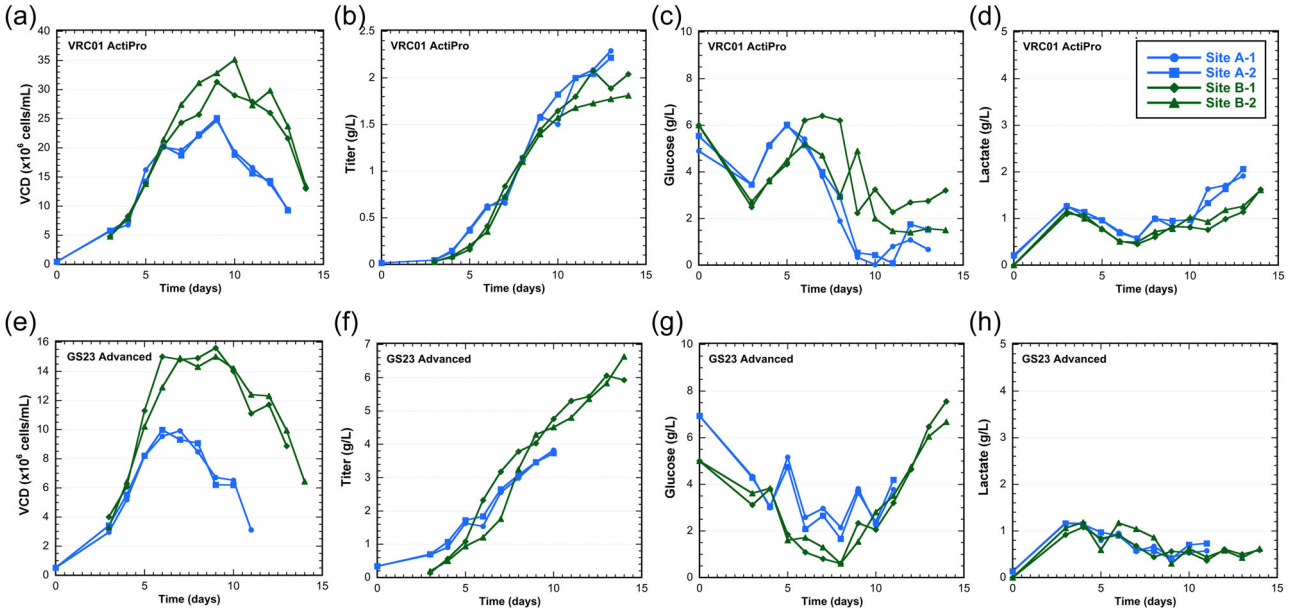

FIGURE 1 Growth characteristic profiles for two cell lines in shake flask fed-batch cultures in respective commercial media. VRC01: (a) VCD; (b) glucose concentration; (c) lactate concentration; and (d) titer. GS23: (e) VCD; (f) glucose concentration; (g) lactate concentration; and (h) titer. Sites A (blue) and B (green), $n = 2$ at each site.

TABLE 2 Key metrics for fed-batch shake flask cultures

	Site A ($n = 2$)		Site B ($n = 2$)		Across sites ($n = 4$)	
	Mean ± SE	RSE	Mean ± SE	RSE	Mean ± SE	RSE
VRC01 in ActiPro						
Max VCD (x10^6 cells/ml)	24.9 ± 0.2	0.80%	33.2 ± 1.9	5.7%	29.1 ± 2.5	8.7%
Max Titer (g/L)	2.25 ± 0.04	1.6%	1.9 ± 0.1	6.8%	2.1 ± 0.1	5.0%
qP (pg/cell/day)	13.1 ± 0.2	1.4%	10.2 ± 0.6	5.4%	11.6 ± 0.8	7.3%
GS23 in EX-CELL Advanced CHO Fed-batch						
Max VCD (x10^6 cells/ml)	9.94 ± 0.03	0.3%	15.3 ± 0.3	2.0%	12.6 ± 1.6	12%
Max Titer (g/L)	3.78 ± 0.05	1.2%	6.35 ± 0.29	4.5%	5.06 ± 0.75	15%
qP (pg/cell/day)	58.65 ± 0.05	0.08%	49.7 ± 0.7	1.3%	54.2 ± 2.6	4.8%

Note: Data for each site and across sites using commercial media are shown. Data reported as means ± standard error (SE) with relative standard error (RSE) shown.

(Klaubert et al., 2021). The lactate concentration profiles were nearly identical, indicating that lactate consumption was achieved, which is the desired outcome in industrial settings (Mulukutla et al., 2014, 2015; O'Brien et al., 2021).

The growth and production profiles for VRC01 enabled cell-specific productivity calculations (Table 2) with example plots of titer as a function of the integrated viable cell density (IVCD) shown in Figure S1A. For Sites A and B, the cell-specific productivities (qP) were 13.1 ± 0.2 and 10.2 ± 0.6 pg/cell/day. Comparison of these values using a *t*-test indicates the cell-specific productivities were significantly different ($p \leq 0.05$) (Table 2). When the data are compared across the sites, the cell-specific productivity is 11.6 ± 0.8 pg/cell/day, yet the RSE was only 7.3%. This low RSE value indicates consistency across sites for the VRC01 cell line, where the site reproducibility was even tighter.

3.1.2 | GS23 performance in shake flasks

Growth characteristics for the shake flask fed-batch cultures for GS23 at both sites using the commercially available media are shown in Figure 1e–h and Table 2. VCD, titer, glucose, and lactate concentration profiles, for both cell lines as shown as duplicate cultures at each site in Figure 1e–h. The commercial medium (EX-CELL Advanced CHO Fed-batch) used a 5% volumetric feed (EX-CELL Advanced CHO Feed 1) every other day. Glucose was

added in parallel to Feed 1 with 150 µl of 45% glucose solution. The GS23 cultures were terminated when viability dropped below 70%. The observed growth profile shapes are similar between the sites despite the difference in absolute maximum VCDs. The VCD at Site A reached approximately 10 million cells/mL, while Site B reached 15 million cells/mL (Figure 1e). Again, the shaking speed was 135 rpm at both sites with different throw diameters and therefore reduced oxygenation likely caused the difference in maximum VCDs.

Despite the differences in maximum VCD between the sites for GS23, the other three metrics—glucose, lactate, and titer profiles were similar across the sites (Figure 1f–h). The pre-determined glucose feeding of 150 µl glucose at Site B maintained the glucose concentration above 1 g/L, while the calculated method used at Site A resulted in glucose depletion. The lactate profiles were consistent between sites. At Site A, the maximum titer of 3.8 g/L was reached on day 10, while the maximum titer at Site B was approximately 6.0 g/L (Day 14); however, the titers are similar between the sites on day 10. The growth and subsequent titer profiles are likely a result of the different incubator throws as discussed earlier (Figure 1b,f) (Klaubert et al., 2021).

The growth and titer profiles for GS23 enabled cell-specific productivity calculations (Table 2) with example plots of titer as a function of the integrated viable cell density (IVCD) shown in Figure S1B. The cell-specific productivities (qP) were 58.6 ± 0.1 and 49.7 ± 0.7 pg/cell/day for Sites A and B, respectively. Comparison of these values using a t-test indicates the cell-specific productivities are significantly different ($p \leq 0.05$), due to the high reproducibility of replicates at each site (Table 2). When the data are compared across the sites, the cell-specific productivity is 54.2 ± 2.6 pg/cell/day and has a 4.8% RSE. This low RSE value indicates consistency across sites for the GS23 cell line, with site consistency even tighter.

These analyses of the VRC01 and GS23 cell lines indicate that while the results from each site were statistically different, the values for cell growth, glucose, lactate, titer, and cell-specific productivity have similar outcomes in the context of biological processes. These data demonstrate that the cell lines have robust growth and consistent cell-specific productivities for the media used for each cell line under shake flask conditions. Based on the success of the shake flask protocols, we sought to expand characterization to controlled scaled-down bioreactors for the two reference cell lines (VRC01 and GS23) in the commercial media and the AMBIC 1.0 media.

3.2 | Bioreactor fed-batch cultivation using two media

Scaled-down bioreactors were used to characterize fed-batch performance across the two cell lines and two media for each cell line. In addition to better DO and pH control, the larger volumes (210 ml working volume) allowed for additional offline/at-line measurements and gas/liquid flow, such as pH, osmolarity, and pCO_2. For simplicity, comparisons of key metrics key growth, and production profiles, will be shown. Other metabolite and cell viability profiles will be presented in Figures S2–S13. The culture conditions and setpoints for each cell line and media are shown in Table 1. All cultures were terminated when viability dropped below 70%. The number of replicate runs, three at Site A and two at Site B were chosen as the minimum required for consistent and predictable culture behavior. Fewer replicates were also chosen to optimize consumables/reagent costs as all experiments were performed within an academic environment. The maximum VCD, maximum titer, number of culture days, and qP, were evaluated within a site and across the sites.

3.2.1 | VRC01 performance in bioreactors

Growth characteristics for the fed-batch bioreactor cultures for VRC01 at both sites using the commercially available media (ActiPro) and AMBIC 1.0 media are shown in Figure 2. As suggested by the cell line generator, the ActiPro bioreactor cultures utilized a pyramid feed schedule with Cell Boost 7a provided at 3% vol. for Days 3–5 and 12–14, 4% for Days 6, 7, 10, and 11, and 5% on Days 8 and 9 with Cell Boost 7b added at one-tenth the volume of Cell Boost 7a. Bioreactor cultures with AMBIC 1.0 media used a daily 3% feed starting on Day 3. VCD and titers are shown in Figure 2, while other metabolite profiles can be found in Figures S2–S7. Glucose was added at the same time as the feeds to reach target values. In the commercial (ActiPro) media, the maximum VCDs were 37.5 and 24.3 million cells/ml at Sites A and B, respectively (Figure 2a). Interestingly, these maximum VCDs are reversed by site when compared to the shake flask cultures (Figure 1a). The increased IVCD at Site A led to higher titers compared to Site B (Table 3 and Figure 2c) with maximum titers of 1.6 and 1.2 g/L for Sites A and B, respectively.

In parallel, VRC01 was cultured in AMBIC 1.0 media (Figure 2) and had significantly lower growth rates and maximum VCD compared to the ActiPro media. Table 3 summarizes these key outcomes for the VRC01 cultures in both ActiPro and AMBIC 1.0 media. For the four key parameters: maximum VCD, culture duration, maximum titer, and cell-specific productivity, the cultures across the sites had relatively consistent behavior as observed by the RSE values below 20% variation.

Consistent with the growth curves presented in Figure 2, cell viability (Figure S2) was maintained above 80% for most of the culture duration. In all cases, cell viability aligned closely across sites (Figure S2). Daily pre-feed glucose readings indicated lower concentrations for Site A in ActiPro media (Figure S3A) with similar behavior for AMBIC 1.0 media (Figure S3B). In all conditions, lactate initially accumulated until approximately Day 3, then the cultures switched to the lactate consumption phase (Figure S3CD). Glutamine and glutamate concentrations are shown in Figure S4, while off-line pH, pO_2, and pCO_2 are shown in Figures S5 and S6. Ammonia and osmolarity profiles are shown in Figure S7, which further demonstrates consistent behavior between replicates and sites. As expected, ammonia increases toward the end of culture

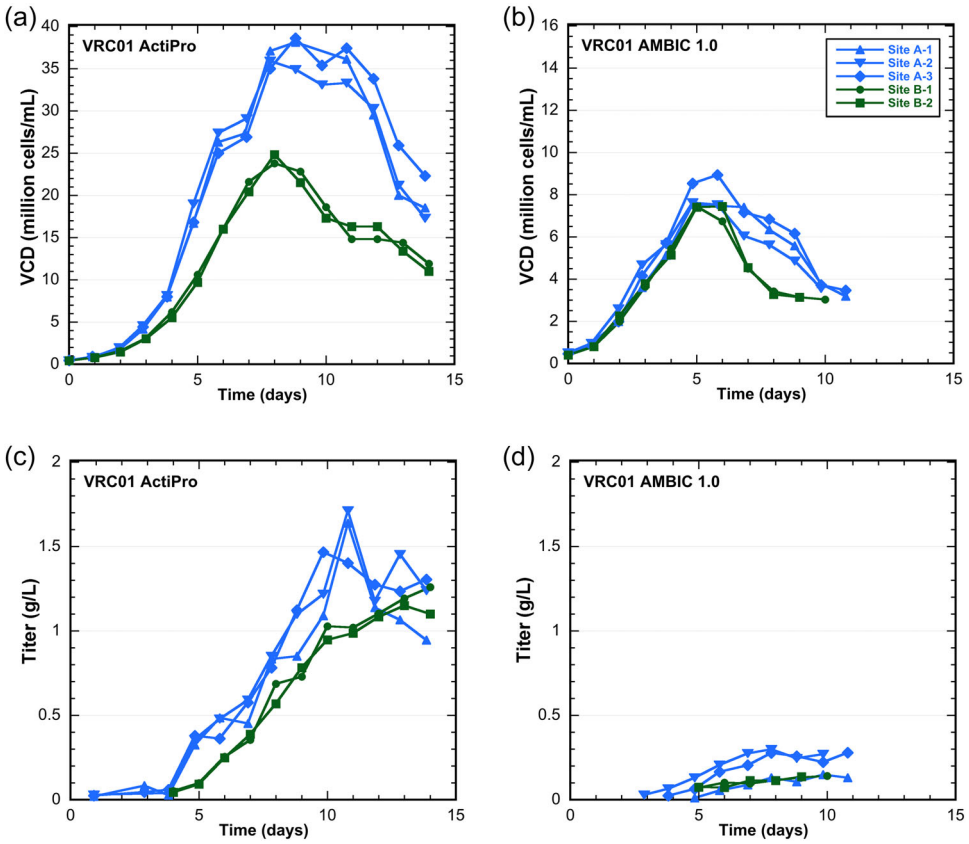

FIGURE 2 Growth characteristic profiles for VRC01 in bioreactor fed-batch cultures in commercial (ActiPro) and AMBIC 1.0 media. ActiPro: (a) VCD and (c) titers. AMBIC 1.0: (b) VCD and (d) titers. Sites A (blue) $n = 3$ and B (green) $n = 2$

TABLE 3 Key metrics for VRC01 bioreactor cultures

VRC01	Site A ($n = 3$) Mean ± SE	RSE	Site B ($n = 2$) Mean ± SE	RSE	Across sites ($n = 5$) Mean ± SE	RSE
ActiPro						
Max VCD (x10^6 cells/ml)	37.5 ± 0.8	2.2%	24.3 ± 0.5	2.1%	32.2 ± 3.3	10%
Culture duration (days)	14.0 ± 0.0	0%	14.0 ± 0.0	0%	14.0 ± 0.0	0%
Max Titer (g/L)	1.60 ± 0.07	4.5%	1.20 ± 0.05	4.5%	1.44 ± 0.11	7.4%
qP (pg/cell/day)	7.77 ± 0.32	4.2%	7.68 ± 0.03	0.4%	7.73 ± 0.18	2.3%
AMBIC 1.0						
Max VCD (x10^6 cells/ml)	8.0 ± 0.5	5.6%	7.4 ± 0.0	0%	7.8 ± 0.3	3.8%
Culture duration (days)	10.7 ± 0.3	3.1%	9.5 ± 0.5	5.3%	10.2 ± 0.4	3.9%
Max Titer (g/L)	0.24 ± 0.05	19%	0.14 ± 0.02	2.6%	0.20 ± 0.04	18%
qP (pg/cell/day)	5.10 ± 1.1	22%	3.74 ± 0.03	0.89%	4.55 ± 0.69	15%

Note: Data are shown for each site and across sites using commercial and AMBIC 1.0 media. Data reported as means ± standard error (SE) with relative standard error (RSE) shown.

corresponding with glutamine and glutamate accumulation contributing to cell death (Figure S7AB). The osmolarity profiles increase for the cultures in ActiPro media due to the high osmolarity of the feeds, while the osmolarity remained constant for the cultures in AMBIC 1.0 (Figure S7C).

These observations provide a set of reference data using both commercial and media generated by the community (AMBIC 1.0) for the VRC01 cell line. These data demonstrated both consistent within-site outcomes (<10% RSE) and acceptable variation between the sites (<20% RSE) for the key parameters: maximum VCD, culture duration,

and maximum titer. The IVCD and titer profiles were used to calculate the qP for each site and media condition (Figure 3). The average qP between the sites was not significantly different ($p > 0.05$). For commercial ActiPro media, Sites A and B had average qPs of 7.8 ± 0.3 and 7.7 ± 0.4 pg/cell/day, respectively. These qP results indicate a successful transfer of the VRC01 process from shake flasks to bioreactors, as cell-specific productivities and titer are comparable to previous literature reports for this cell line and media (Chitwood et al., 2021; Synoground et al., 2021). For the AMBIC 1.0 media, cell-specific productivities and titers were significantly lower than in ActiPro. The AMBIC 1.0 media is a leaner media with respect to amino acids in the media and feeds. In AMBIC 1.0 media, the qPs were 5.1 ± 1.9 and 3.7 ± 0.1 pg/cell/day for Sites A and B, respectively. The qP in the AMBIC 1.0 media was not different between the sites ($p > 0.05$). The qP averages across the sites ($n = 5$) were 7.74 ± 0.18 pg/cell/day in ActiPro and 4.55 ± 0.69 pg/cell/day in AMBIC 1.0 where the RSE values were low (Table 3).

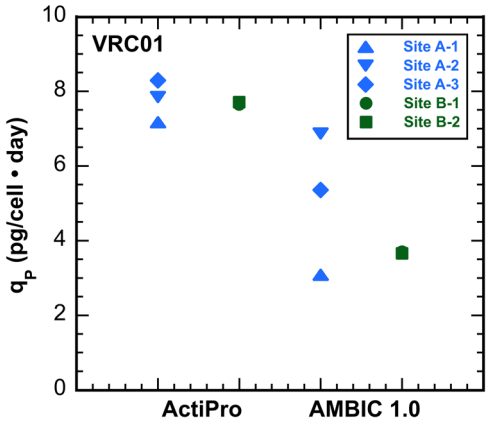

FIGURE 3 Cell specific mAb productivity for VRC01 from bioreactor cultures in commercial (ActiPro) and AMBIC 1.0 media. Site A (blue) $n = 3$ and Site B (green) $n = 2$.

3.2.2 | GS23 performance in bioreactors

Growth characteristics for the fed-batch bioreactor cultures for GS23 at both sites using the commercially available media (EX-CELL Advanced) and AMBIC 1.0 media are shown in Figure 4. VCD and titers are shown in Figure 4, while other metabolite profiles can be found in Figures S8–S13. For both the commercial media (EX-CELL Advanced) and AMBIC 1.0 media a 5% volumetric feed was added every other day as described in the Materials and Method section and outlined in Table 1. Glucose was added at the same time as the feeds to reach target values with cultures terminated when viability

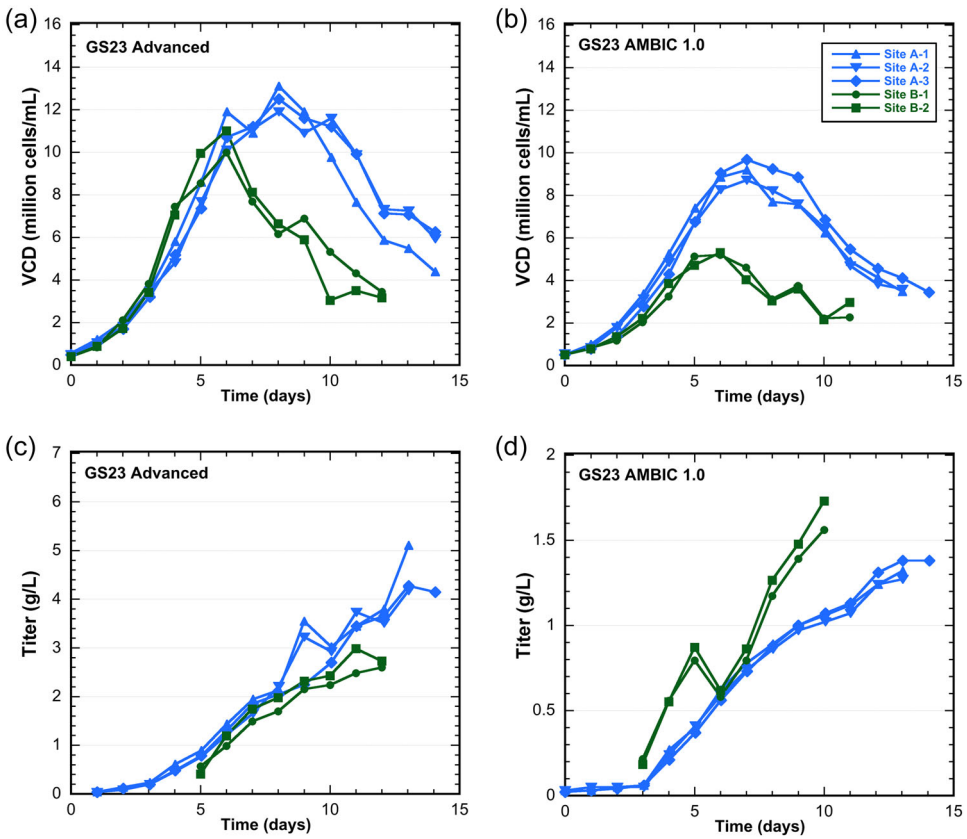

FIGURE 4 Growth characteristic profiles for GS23 in bioreactor fed-batch cultures in commercial (Advanced CHO Fed-Batch) and AMBIC 1.0 media. Advanced: (a) VCD and (c) titers. AMBIC 1.0: (b) VCD and (d) titers. Site A (blue) $n = 3$ and Site B (green) $n = 2$

TABLE 4 Key metrics for GS23 bioreactor cultures. Data are shown for each site and across sites in commercial and AMBIC 1.0 media. Data reported as means ± standard error (SE) with relative standard error (RSE) shown.

	Site A (n = 3)		Site B (n = 2)		Across Sites (n = 5)	
GS23	Mean ± SE	RSE	Mean ± SE	RSE	Mean ± SE	RSE
EX-CELL Advanced CHO Fed-Batch						
Max VCD (x10^6 cells/mL)	12.5 ± 0.4	2.8%	10.5 ± 0.5	4.9%	11.7 ± 0.6	4.7%
Culture duration (days)	13.3 ± 0.3	2.2%	12.0 ± 0.0	0%	12.8 ± 0.4	2.9%
Max Titer (g/L)	4.52 ± 0.29	6.5%	2.80 ± 0.19	6.9%	3.8 ± 0.5	12%
qP (pg/cell ·day)	44.4 ± 1.4	3.2%	42.5 ± 2.3	5.6%	43.7 ± 1.2	2.7%
AMBIC 1.0						
Max VCD (x10^6 cells/mL)	9.2 ± 0.3	3.0%	5.3 ± 0.1	1.0%	7.6 ± 1.0	13%
Culture duration (days)	13.3 ± 0.3	2.5%	11.0 ± 0.0	0%	12.4 ± 0.6	4.8%
Max Titer (g/L)	2.61 ± 0.13	5.0%	1.65 ± 0.85	5.2%	2.22 ± 0.25	11%
qP (pg/cell ·day)	35.7 ± 0.6	1.7%	40.1 ± 1.9	4.8%	37.4 ± 1.3	3.5%

dropped below 70%. In the commercial media, the maximum VCDs were 12.5 million cells/ml and 10.5 million cells/ml at Sites A and B, respectively. Consistent with the observations for the VRC01 cell line in bioreactors, these maximum VCDs are reversed by site when compared to the shake flask cultures (Figure 1e). The increased IVCD at Site A led to higher titers compared to Site B (Table 3 and Figure 4c). The final titers were 4.5 g/L and 2.8 g/L for Sites A and B, respectively. The cell viability cutoff of 70% was reached at Site B on Days 12 and 14 at Site A (Figure S9).

In parallel, GS23 was cultured in AMBIC 1.0 media (Figure 4) and had significantly lower growth rates and maximum VCD compared to the EX-CELL Advanced media. Table 3 summarizes these key outcomes for the GS23 cultures in both EX-CELL Advanced and AMBIC 1.0 media. Overall, the cultures across the sites had relatively consistent behavior as observed by the RSE values below 20% variation.

Daily glucose readings were similar for the sites (Figure S8A) with even more consistent behavior in the AMBIC 1.0 media (Figure S8B). In all conditions, lactate initially accumulated until approximately Day 3 when the cultures switched to the lactate consumption phase (Figure S8CD). Interestingly, lactate reached significantly higher levels at Site B which corresponded to shorter culture duration at Site B as shown by cell viability in Figure S9. Glutamine and glutamate concentrations are shown in Figure S10, while off-line pH, pO_2, and pCO_2 are shown in Figures S12 and S13. Ammonia and osmolarity profiles are shown in Figure S11, which further demonstrate consistent behavior between replicates and sites. As expected, ammonia increases toward the end of the culture (Figure S10A,B). The osmolarity profiles were constant for both the EX-CELL Advanced and AMBIC 1.0 media due to the physiological osmolarity of these feeds (Figure S13C,D). These metabolite observations shed additional light on performance across sites and highlight the challenges in generating parallel data at multiple locations. One chooses which parameters are critical for process alignment while permitting others to vary more widely.

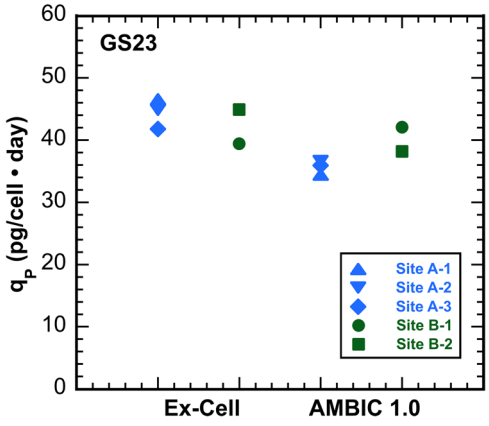

FIGURE 5 Cell specific mAb productivity for GS23 from bioreactor cultures in commercial (Advanced CHO Fed-Batch) and AMBIC 1.0 media. Site A (blue) $n = 3$ and Site B (green) $n = 2$

These GS23 culture observations provide a set of reference data using both commercial and media generated by the community. These data demonstrated both consistent site outcomes (<10% RSE) and acceptable variation between the sites (< 20% RSE) for the key parameters: maximum VCD, culture duration, and maximum titer (Table 4). The IVCD and titer profiles were used to calculate the qP for each site and media condition (Figure 5). For commercial EX-CELL Advanced media, Sites A and B had average qPs of 44.4 ± 1.4 and 42.5 ± 2.3 pg/cell/day, respectively. The average qP between the sites was not significantly different ($p > 0.05$). These qP results indicate a successful transfer of the GS23 process from shake flasks to bioreactors, as cell-specific productivities and titer are comparable to the data provided by the cell line originator. For the AMBIC 1.0 media, cell-specific productivities and titers were significantly lower than in EX-CELL Advanced media. The qPs for GS23 in the AMBIC 1.0 media were 35.7 ± 0.6 and 40.1 ± 1.9 pg/cell/day for Sites A and

B, respectively. The qPs in the AMBIC 1.0 media were not different between the sites ($p > 0.05$). The qP averages across the sites ($n = 5$) were 43.7 ± 1.2 pg/cell/day in EX-CELL Advanced and 37.4 ± 1.3 pg/cell/day in AMBIC 1.0 where the RSE values were low, as shown in Table 4. As observed previously for VRC01, the AMBIC 1.0 media results in slightly lower qP compared to the corresponding commercial media; however, in both media, reproducible and consistent results were achieved for key growth and production parameters as highlighted in Table 4.

3.2.3 | Round Robin

The industrial community is very familiar with the challenges inherent to technology transfer across sites and between organizations. The work here prompted the development of effective documentation to assist in technology transfer between academic researchers. Even slight differences in protocols were initially observed to impact the consistency of data across sites. For example, tasks including steps from vial thaw and expansion to feed storage during culture had to be aligned. Bioreactor control schemes for pH and DO were shared across sites as well as temperature setpoints including media conditioning before inoculation. Even with significant efforts towards alignment across the entire process, the variations between sites may be attributable to changes in culture media between runs and minor differences in equipment setup. Also, titer alignment was required due to instrumentation differences between the sites.

To reconcile titer measurement differences, samples were exchanged between sites (a round-robin). The supporting information file details the round-robin titer data analysis with Cedex and Octet instruments. In the end, both instruments provided self-consistent data, and the data were correlated between the sites (Figure S14). For simplicity, the Octet equivalent titers were presented for the two reference cell lines (Figures 1, 2, and 4). Overall, the experimental matrix considered two cell lines and two media types at two locations, to generate datasets with online and offline measurements. These datasets provide an expected range of culture behavior and can be used as a benchmark for those who wish to work with these cell lines and media in shake flasks or scaled-down bioreactor systems. These results describe, for the first time, industrially relevant CHO cell lines that will be made available to the broader research community for noncommercial use. This information will serve as a foundation for additional studies to expand the utility of these reference cell lines, especially in a manner available to both the academic and industrial communities. In addition, we provide a detailed description of an open-source industrially relevant, chemically defined cell culture media that may be used by the community.

In conjunction with the work presented here, the VRC01 cell line has a growing body of literature as a reference platform for the CHO biopharmaceutical industry. Recent publications have highlighted the downregulation of host cell proteins with cell age (Hamaker et al., 2022), safe harbor locations for genetic editing (Hilliard & Lee, 2021), and the impact of ammonia stress on glycosylation (Synoground et al., 2021) within this cell line. Maintenance of production stability during long-term culture is also an important parameter for biopharmaceutical manufacturing and has been previously studied in the VRC01 cell line (Hamaker et al., 2022). The qP of the VRC01 cell line drops at each increment of 30 generations (Hamaker et al., 2022). These previously published results suggest that cryopreservation and production experiments should be limited to early generation cultures. Although higher titer and productivity is achievable in the GS23 cell line, the VRC01 line also represents a useful reference cell line for standardization across industry and academic laboratories.

4 | CONCLUSIONS

The performance for two cell lines in two different media was compared at two different culture scales. It was observed that maximum VCD and titers varied between the sites; however, the qPs were not significantly different between the sites for the scaled-down bioreactor conditions. Despite slightly lower titers and productivity observed in bioreactors as compared to shake flasks, both CHO cell lines demonstrated robust growth and performance characteristics in each culture scale. These datasets and protocols will enable the biomanufacturing community to compare process improvements via (1) two reference cell lines with industrially relevant performance; (2) crowd-sourced chemically defined media; (3) cultivation protocols for these cell lines; and (4) availability of reference datasets for shake flask and bioreactor cultures.

AUTHOR CONTRIBUTIONS

Lauren T. Cordova, Hussain Dahodwala, Kathryn S. Elliott, Jongyoun Baik, Daniel C. Odenewelder, Douglas Nmagu, Bradley A. Skelton, Lisa Uy, Stephanie R. Klaubert, Benjamin F. Synoground, Dylan G. Chitwood, Venkata Gayatri Dhara, Harnish Mukesh Naik, and Caitlin S Morris contributed to the collection of data. Seongkyu Yoon, Michael Betenbaugh, Jon Coffman, Frank Swartzwelder, Michael P. Gillmeister, Sarah W. Harcum, and Kelvin H. Lee conceived the project and directed the project. Lauren T. Cordova, Sarah W. Harcum, and Kelvin H. Lee finalized the writing of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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