Comparative study for the production of monoclonal antibodies in single-use vs stainless steel bioreactors based on product quality and stress factor

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Abstract
Single-use bioreactors (SUB) and stainless steel bioreactors (SSB) differ in their physical properties. This can have an effect on the performed processes. To be able to guarantee flexible planning in a facility, any kind of processes in SUB and SSB must be sufficiently similar. To date, assessments of SUB and SSB have been exclusively specific for one single product. This study shows a general nonproduct-specific comparison of the production of different types of monoclonal antibodies in different scales of SUBs and SSBs. The study is set up with 72 clinical manufacturing fermentation runs in total by comparing the cell culture performance and quality attributes of these two bioreactor systems. The outcome is that SUBs and SSBs can produce monoclonal antibodies with comparable cell culture performance and product quality. This general comparability paves the way for SUBs to produce new and existing products for (clinical) manufacturing.

KEYWORDS
bioreactor, comparison, fermentation, monoclonal antibody, single-use, stainless steel

1 | INTRODUCTION

In the current fast-changing field of biopharmaceutical production, there is a strong trend toward intensification and continuous manufacturing.\(^1\)\(^2\) As a result it is mandatory to increase productivity, capacity, product titer (PRO), and flexibility. Concurrently the complexity, footprint, investment and costs should be decreased\(^3\)\(^4\) in order to remain competitive. The use of single-use bioreactor (SUB) systems can cope with most of these challenges in modern biomanufacturing facilities.

Some of the advantages of SUB equipment are lower capital expenditure, smaller footprints, and faster implementation times than traditional stainless steel bioreactor (SSB) systems.\(^5\)\(^6\) Consequently, it is not surprising, that single-use technologies represent the state of the art in modern bio-manufacturing.\(^7\)

To implement single-use technologies in a modern facility and to benefit from the above-mentioned numerous advantages, it is very important to show comparability of SSBs and SUBs. The comparison should comprise product quality and cell culture performance, to pave the way for SUBs in clinical manufacturing and beyond.

Although SUBs are state of the art, existing comparison studies in the literature just provide information about SSBs and SUBs being comparable relating to only one product.\(^8\) This means that a new study is required for every new product entering clinical and/or commercial manufacturing.
In this study a general comparability with an equivalence test of SSB and SUB was performed in a multiproduct GMP facility based on product quality and cell culture performance, independent of products and manufacturing processes. The study illuminates that SSBs and SUBs can produce monoclonal antibodies (MABs) with comparable quality in general. Thus, no further studies are required for existing and new products. Hence, the interchangeable equipment utilization of various processes enables a highly flexible manufacturing without additional work for bioreactor selection.

2 METHODS AND MATERIAL

2.1 Facility setup

The comparison of SSB and SUB was performed in a multi-equipment GMP facility that offers a wide range of different types of bioreactor systems and scales. In this GMP facility, material for clinical Phase 1 to Phase 3 is produced on different process platforms. In order to facilitate the production planning in the GMP facility, a data analysis of 10 different products (see Figure 1 and Section 2.2) at different scales (production fermentation: 250 L and 1000 L volume) and in different bioreactor systems (SSBs and customized SUBs) was conducted to demonstrate that processes can either use the SSB or the SUB.

2.2 Examined products and SSB/SUB data in total

The data for the SSB/SUB comparison comprises 10 different products on three different process platforms. A process platform was characterized by using the same media components and process parameters during fermentation. The first process platform used for the comparison included two of the 10 analyzed products. The second platform contained five products while the third platform contained the final three products. The bioreactor scale for all the examined production fermentation runs varied in volume between 250 and 1000 L. For each of the 10 different products, fermentation runs were performed in either the SSB or the SUB. In total 72 fermentation runs under GMP conditions were observed: 31 in the SSB and 41 in the SUB (see Figure 1).

Criteria for the comparison (product attributes) are listed in Section 2.3.

The sample management used for comparison can be found in section 9.1 of Appendix S1.

2.3 Criteria for the comparison: Product attributes

Prior to data analysis, the seven most suitable product attributes were chosen to perform the comparison (details see sections 9.2-9.8 of Appendix S1 and Table 2). With these attributes the cell culture performance and the product quality in the SUBs and the SSBs were compared. An equivalence acceptance criteria (EAC) was defined for each of the selected attributes that were considered acceptable for the comparison.

**FIGURE 1** Total data basis for the stainless steel and single-use bioreactors (SSB/SUB) comparison. In total 72 fermentation runs under GMP conditions out of 10 different products (SSB runs: 31; SUB runs: 41), performed on three different process platforms were conducted for comparison.
The cell culture performance in the different bioreactor systems was examined over five cell culture performance indicators (CCPI): PRO with an EAC range (EACR) of ±20%, the specific productivity (qp) with an EACR of ±20%, the viability at harvest with an EACR of −10%, the lactate dehydrogenase activity (LDH) at harvest with an EACR of ±20% and the maximum viable cell density (VCD\textsubscript{max}) with an EACR of ±20%.

Additionally, product quality attributes were evaluated. First was purity measured by size exclusion chromatography (SEC) with an EACR of ±4 area-%. Second was purity measured by ion exchange chromatography (IEX) with an EACR of ±5 area-%.

Based on these attributes, if a process performs within these specifications concerning cell culture performance and product quality, both bioreactor systems can produce biopharmaceuticals in the same quality.

### 2.4 Statistical methods

To show comparability of the two datasets of SSB and SUB, an equivalence test based on the 2 one-sided test (TOST) procedure from Schuirmann\(^9\) and Westlake and Kirkwood\(^10\) was performed for each attribute. This statistical test demonstrates equivalence when the obtained data provides enough evidence that the absolute difference in means is not larger than the prespecified EACR. The EACR is defined by subject matter experts in advance considering all relevant aspects including the given method variability, release specification criteria/limits, and historical manufacturing data. A true difference within the EACR (−EAC, +EAC) is considered not meaningful.

In the TOST the observed mean difference between the two groups with the respective 90% confidence interval is computed and compared with the EACR, resulting in four possible categories (see Figure 2).

The two datasets, SSB and SUB, are additionally affected by a covariate: the product. Thus, the equivalence test is not calculated on the mean difference of SSB and SUB, but on the difference of the least-squares means (LSMeans). The LSMeans will be estimated based on a linear regression model:

\[
Y = \beta_0 + \beta_1 \cdot X + CoX + \epsilon, \tag{1}
\]

where \(Y\) is the dependent variable, \(X\) is the independent variable, and \(CoX\) is the covariate. The \(\beta_i\), \(i = 0, 1\) denote the coefficients to be estimated and \(\epsilon\) represents the estimated error.

The LSMeans reflects the covariate-adjusted means for the two groups and thus the equivalence test is based on the differences of the LSMeans and the corresponding 90% confidence intervals. As a consequence, the two datasets SSB and SUB are equivalent when the difference in the product-adjusted LSMeans with the respective 90% confidence interval is completely contained in the EACR (−EAC, +EAC) (see Figures 1A and 2).

**FIGURE 2** Equivalence test result categories (normalized to equivalence acceptance criteria). (1a) Equivalent: The observed mean difference and the 90% confidence interval are completely within the equivalence acceptance criteria range (EACR). (1b) Equivalent in Sample Mean Only: The observed mean difference is within the EACR; however, the 90% confidence interval is partially outside the EACR. (2a) Failed-to-be-Equivalent: The observed mean difference is outside the EACR and the 90% confidence interval is partially outside the EACR. (2b) Inequivalent: The observed mean difference and the 90% confidence interval are completely outside the EACR. Notes: red boxes = mean difference, bars = 90% confidence interval of mean difference, black line = line of zero difference, dotted lines = normalized lower and upper equivalence acceptance criteria.
Since the variability of the responses PRO, qP, viability at harvest and the LDH are dependent on the mean, a log-transformation (natural logarithm) is applied prior to data analysis. Accordingly, the EACR of ±20% is defined as \((\frac{1}{\log(1.2)}, \log(1.2))\).

All calculations are performed in R\(^1\) with the package lsmeans or in JMP.\(^12\)

To illustrate the results of the equivalence test, the EACR for each attribute was normalized to −1 and +1.

### 3 RESULTS

#### 3.1 Predefined assumptions for comparison

Eight predefined assumptions were made (see Table 1) prior to data screening, evaluation, and defining the product attributes (see Section 2.3 and Table 2). The purpose of these predefined assumptions was to compare SSB and SUB fermentation runs in a representative and statistically correct way.

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| Predefined assumptions |
|-------------------------|
| 1 Important product attributes of SSB and SUB were compared independently of each product (see Section 3.2) |
| 2 Important product attributes including their EACRs were defined before looking at data for the SSB/SUB comparison (see Section 2.3) |
| 3 For each product attribute the SSB fermentation data is used as reference |
| 4 Similar variance for the evaluated products and scales were assumed |
| 5 The SSB racks used for fermentation are comparable to each other |
| 6 The SUB racks used for fermentation are comparable to each other |
| 7 The chosen products are representative for actual and future production of monoclonal antibodies, because different antibody formats were examined |
| 8 Seed- and inoculum trains were out of scope |

Abbreviations: EACR, equivalence acceptance criteria range; SSB, stainless steel bioreactor; SUB, single-use bioreactor.

### TABLE 1 List of the predefined assumptions that were defined before looking at the data to compare SSB and SUB fermentation runs. These assumptions were made so that a statistically valid and representative comparison of SSB and SUB fermentation runs could be conducted independent of product and scale. These assumptions were established before the product attributes (see Table 2) were analyzed

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Abbreviations: EACR, equivalence acceptance criteria range; SSB, stainless steel bioreactor; SUB, single-use bioreactor.

### TABLE 2 Product attributes and respective EACRs for comparing the SSB with the SUB. The seven product attributes are subdivided into attributes affecting the product quality and cell culture performance indicators CCPIs (for details see section 9.2-9.8 of Appendix S1 and Section 2.3). Each attribute has a respective EACR (for description see Section 2.4) to give the acceptance range for the subsequent analysis by equivalence test (see Section 3.3).

| Product attribute | EACR |
|-------------------|------|
| **Product quality** | |
| 1 Purity (SEC) (Area-%) | ±4 Area-% |
| 2 Charge distribution (IEX) (Area-%) | ±5 Area-% |
| **CCPI** | |
| 3 PRO (@ Harvest) (g/L) | ±20% |
| 4 Spec. Productivity (qP) (pg/(cell/days)) | ±20% |
| 5 Viability (@ Harvest) (%) | −10% |
| 6 LDH (@ Harvest) (U/L) | ±20% |
| 7 VCD\(_{\text{max}}\) (x10\(^5\) cells/mL) | ±20% |

Abbreviations: CCPI, cell culture performance indicators; EACR, equivalence acceptance criteria range; LDH, lactate dehydrogenase activity; SSB, stainless steel bioreactor; SUB, single-use bioreactor.
Section 2.3) are product independent. This ensures that SSB and SUB can be compared in general and independently of a single product. (ii) The relevant product attributes, including their EACRs, have to be defined before taking an initial evaluation of the already existing data, to avoid being biased. (iii) The SSB data is set as a reference for comparison. (iv) Similar variances were assumed for the raised data and the different bioreactor scales. (v) The different SSBs are comparable to each other. (vi) The different SUBs are comparable to each other. As a consequence of (v) and (vi) it is possible to collate SSB and SUB data. (vii) Due to the examination of different antibody formats, the chosen products are representative for actual and future productions of MABs. (viii) The seed- and the inoculum trains are out of scope. This implies for the considered comparability of the production bioreactor data, that these earlier process steps are comparable as well.

With these assumptions the right criteria for a representative and statistically correct comparison of SSBs and SUBs were chosen prior to data inspection.

### 3.2 Descriptive analysis

The first step in the data analysis was to examine the product-specific influence on the product attributes (as described in Section 2.4). These attributes are divided into attributes concerning product quality and the CCPI in the SSB and the SUB (see Section 2.3). To depict the product-specific influence, data were descriptively outlined in a scatter plot (see Figures 3-5).

**FIGURE 3** Analysis of product attributes by product (1/3). For each product attribute a product-specific analysis was done to evaluate the product specific influence on the attribute. The figures show that there can be a huge difference between the product-specific means (short green lines) and thus the data has to be normalized to a common mean (grey line), to be able to compare the attributes of all 10 products with the same equivalence acceptance criteria range. The figures also display that the stainless steel bioreactor (SSB) vs the single-use bioreactor (SUB) data are located quite close within the products (SSB [stars] and SUB [filled circles]). This implies an initial comparability of the two bioreactor systems.
FIGURE 4  Analysis of product attributes by product (2/3). For each product attribute a product-specific analysis was done to evaluate the product-specific influence on the attribute. The figures show that there can be a huge difference between the product-specific means (short green lines) and thus the data has to be normalized to a common mean (grey line), to be able to compare the attributes of all 10 products with the same equivalence acceptance criteria range. The figures also display that the stainless steel bioreactor (SSB) vs the single-use bioreactor (SUB) data are located quite close within the products (SSB [stars] and SUB [filled circles]). This implies an initial comparability of the two bioreactor systems.

The data shows a huge variability between the 10 different products for every product attribute. This means, that the product-specific influence is high and has to be taken into account (see Section 2.4).

To give an example, PRO varies for all 10 products between arbitrary values of 0.1 and 0.9. However within one product, the PRO values for the SSB and the SUB are fairly consistent and vary considerably less. This indicates that the difference is independent of the used bioreactor system (SSB or SUB).

As seen in Figures 3 to 5 there are some single data points that are more extreme than the rest of the data (eg, PRO of product 04 produced in the SUB in Figure 3). As this happens to be single events only and thus are not generally related to the used bioreactor system, they were included in the analysis. Additionally, it is not recommended to exclude single data points, because all data used in the analysis belong to batches that were released after production of the cell culture.

To show a general comparability of SSB and SUB a TOST was then performed and the product was taken into account as a covariate (for description see Section 2.4 and for results see Section 3.3).

3.3  Equivalence test results

The overall result of the equivalence test (for description see Section 2.4) is, that all predefined attributes show comparability (equivalence) of SSB and SUB (see Figure 6).

In more detail, all product quality attributes (purity: SEC_{HMW}, SEC_{LMW}, SEC_{Monomer}, and charge distribution: IEX_{acidic}, IEX_{basic}, and IEX_{main}) are classified as “equivalent” (category 1a, see Figure 2). Four out of five CCPIs are also classified
FIGURE 5  Analysis of product attributes by product (3/3). For each product attribute a product-specific analysis was done to evaluate the product specific influence on the attribute. The figures show that there can be a huge difference between the product-specific means (short green lines) and thus the data has to be normalized to a common mean (grey line), to be able to compare the attributes of all 10 products with the same equivalence acceptance criteria range. The figures also display that the stainless steel bioreactor (SSB) vs the single-use bioreactor (SUB) data are located quite close within the products (SSB [stars] and SUB [filled circles]). This implies an initial comparability of the two bioreactor systems.

FIGURE 6  Result of the equivalence test. The figure shows the final result of all product attributes taken into account for the stainless steel/single-use bioreactors (SSB/SUB) comparison. The space between the dotted lines stands for the equivalence acceptance criteria range (EACR) (see Section 2.4) of the product attribute listed on the right. The range is normalized to −1.0 and +1.0 to display all the attributes in one graph. The ground line in the middle stands for zero difference in the mean between the SSB and the SUB data. The red squares indicate the mean difference between SSB and SUB data corrected by the product impact. The whiskers show the 90% confidence interval applied to their respective differences (for explanation see Section 2.4). In summary, for every attribute the mean difference is smaller than the predefined EACRs. The confidence interval lies completely within the dotted lines for all attributes except LDH. For LDH the outcome of the comparison is equivalent in sample mean only, since the confidence interval ranges out of the EACR. All other attributes are ranked as equivalent.
as “equivalent” (PRO, qP, viability, and VCD_{max}). Only one attribute, LDH, is classified as “equivalent in sample mean only” (category 1b, see Figure 2).

For every product quality attribute and CCPI, except LDH, the difference between the SSB and the SUB lies within the predefined EACR including their confidence intervals (see Section 2.3).

For LDH, the mean difference lies within the predefined EACR, but the lower limit of the 90% confidence interval is out of range.

To gain this result, all used data was compiled and treated as described before (see Section 2.4).

To sum up, SSBs and SUBs are equivalent and thus comparable in general concerning product quality and cell culture performance.

4 | DISCUSSION

This study focuses on a general comparability of SSB and SUB based on product quality and cell culture performance, independent of products produced with the systems and manufacturing processes applied in a multiproduct GMP facility.

To give evidence, the data from 72 fermentation runs (31 in the SSB, 41 in the SUB) was statistically evaluated with an equivalence test (see Section 2.4).

As a result, all product quality attributes and most of the CCPIs meet their EAC for the SSB/SUB comparison on average.

According to the defined result classifications in Figure 2, all product quality attributes are equivalent. Concerning cell culture performance, four out of five CCPIs are equivalent. LDH is the only indicator that was classified as equivalent in sample mean only. This was caused by the initial use of nonideal internal fittings and fermentation parameters in the SUB for the first runs in the single-use system. However, incremental improvement of both the fittings (gassing unit) and the parameters alleviated this problem.\(^{13}\)

To go more into detail, the LDH is slightly higher in the SUB than in the SSB, but the mean difference lies within the EACRs. For a better understanding and a more precise analysis, the LDH was compared for all 10 products for the SSB and SUB (see Figure 7). This resulted in wide variation of the LDH value within each product, independent of which bioreactor system was used.

This means that it is difficult to compare the LDH in the two bioreactor systems for all 10 products together.

For each product itself, there is no real difference between the LDH in the SSB and the SUB, except for product 09. Product 09 was the first product produced in the SUB system in the facility that was used for the comparison. Due to a lack of experience with the system, SUB fermentation data for LDH is higher than the SSB data for this product.

**Figure 7** Data basis for LDH-classification. This figure shows why LDH was classified as “equivalent in sample mean only”. LDH varies considerably within each product (especially for product 01, 02 and 05), so the 90% confidence interval gets very wide. The high intra-product variation is not caused by the different bioreactor types SSB (stars) and SUB (filled circles).
The reason for the increased LDH data in the SUB is the usage of nonideal fermentation settings and parameters used in the first two of three performed supplies (more than two batches produced in a row) of product 09 (see Figure 8).

Within these three supplies, different gassing units and settings were applied. In each supply a decrease in the LDH value in the SUB was achieved. After the first supply an open pipe was used for gassing instead of a microsparger unit. After the second supply an adjustment of the gas flow rate and the stirrer speed was performed. This new SUB system setup eliminates smaller bubbles during gassing and consequently reduces cell damage during fermentation. Subsequently, the LDH value and therefore the fermentation settings and fittings with this final approach were comparable to the SSB runs and were applied to all other nine products.

Besides the discussed LDH topic, a product-specific influence on the evaluated product attributes was examined (see Section 3.2). This examination showed a huge difference in the value of an attribute between the 10 different products; however, the data showed no difference between SSB and SUB within each product. Consequently, there were large differences between the observed 10 products independent of which bioreactor system the fermentation was performed in.

All 72 fermentation runs used were released after production. Even if there has been issues raised during production, they were evaluated as not relevant, because no impact on product quality or process performance was observed.

The main reason for having a combined analysis in this study is the aim to show the interchangeability of SSB and SUB independent of the product. The interest is not in the differences caused by the various products (there can be big differences in mean) but only in the mean difference between the two systems.

Additionally, the small sample size for the majority of product-specific datasets (eg, product 06 only has two SSB and three SUB data points) makes it impossible to perform product specific equivalence tests.

In general, the variabilities between the two groups of interest (SSB and SUB) are comparable (ie, if a product has larger variability in general this is also reflected in the variabilities of both groups separately). However, for some product quality attributes different variabilities were observed (heteroscedasticity). In scenarios with heteroscedasticity the 90% confidence interval might be slightly biased. Nevertheless, since all product attributes except LDH are well within the EACR, this slight inaccuracy is negligible and does not change the final conclusions.

The scope of the study is a general comparison of the two established systems (SSB and SUB), which is not related to specific geometries or internal fittings. In this study 10 different products freely allocated to both bioreactor systems were taken into account and give evidence, that MABs can be produced with comparable product quality and process performance in both systems.

As a consequence, both established bioreactor systems can be used interchangeable and no specific testing for every single product is needed.

When introducing a new bioreactor type into the facility, which is of the same manufacturing type than an already existing bioreactor (including internal fittings), only one single successful comparison is needed to demonstrate interchangeability.

**FIGURE 8** Reason for lactate dehydrogenase activity (LDH) being “equivalent in sample mean only” for product 09 (see Figure 7). The figure shows the LDH data of product 09 divided into different adaptions made during the production processes (stainless steel bioreactor, SSB [stars] and single-use bioreactor, SUB [filled circles]). This product was the first ever produced in the SUB system (in the used facility), thus there were some adjustments made after each supply. The first supply was performed with a microsparging unit for the gassing in the SUB. Before the second supply the microsparging unit was replaced through an open pipe gas inlet (leading to impact on the LDH see Section 4). The usage of the open pipe shows a decrease of the LDH in the SUB. For the third supply, the gas flow rate and the stirrer speed were adjusted to be more comparable to the SSB. As a consequence of this adjustment, the mean LDH in the SUB shifted to become similar to the mean LDH in the SSB.
When a new system (eg, SUB) is introduced into a facility with an already existing system (eg, SSB), a first detailed comparison between the two systems needs to be performed. If comparison between the two systems can be demonstrated, this study can be applied for all further products to use the two systems interchangeable.

This case also applies for introducing a new bioreactor type, which is different to the already existing manufacturing types (eg, different geometry or internal fittings).

A good example for this detailed comparison is described earlier in this chapter, where the sparging device has to be changed to achieve comparability. The initially used sparging device in the SUB caused more stress to the cells and thus LDH was not comparable to the SSB runs. Consequently, the sparging device in the SUB was changed to an open pipe sparger for all future fermentation runs to reduce cell stress and therefore to allow comparable production to the SSB concerning LDH.

Afterwards you can produce every desired product with comparable product quality and process performance to your existing equipment in your newly introduced equipment (SSB or SUB).

The particular bioreactor type and internal fittings needed for a successful comparison of the new equipment should be dependent on the geometry and the internal fittings of the already existing equipment.

In summary, all selected attributes for the general comparison of SSB and SUB are equivalent. Thus, the product quality and the cell culture performance in the SUB, independent of product and process platforms, are comparable to the SSB. Therefore, the usage of SUBs in clinical manufacturing is an opportunity to be more flexible and adaptable to future needs and techniques (eg, perfusion or high cell density processes).

5 | CONCLUSION

This study demonstrates that SSBs and SUBs can be treated as equivalent since they produce products with comparable product quality and cell culture performance (see Section 4). In the current literature, there are just comparisons of SSBs and SUBs concerning one single product available. This means that for each product that was produced in SSBs so far and should be transferred into a SUB in the future, a new comparability study has to be performed in advance (SUB to SSB as well). The equivalence study of this work can be used as a reference for every present and future production process and antibody format to produce biopharmaceuticals for clinical manufacturing in SSB likewise SUB equipment under GMP conditions without any further studies needed concerning the usage of the bioreactor system.

The requirement for applying this study in general is to perform at least one successful comparison of SSB and SUB in a facility.

For implementation of new equipment (SSB or SUB) in a facility, there is just an initial comparison with the other existing equipment needed. The specific bioreactor type and the specific internal fittings of the new equipment should enable a comparable production with the existing equipment. Afterwards the new equipment can be used without the need of further studies as well.

With this study, the way is paved for using the advantages of SUBs (eg, increase productivity, lower capital expenditure, flexibility, smaller footprints, and faster implementation times) in place of SSBs, so as to remain competitive as a manufacturer in biomanufacturing without the need of further comparison studies.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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REFERENCES

1. Fisher AC, Kamga M-H, Agarabi C, Brorson K, Lee SL, Yoon S. The current scientific and regulatory landscape in advancing integrated continuous biopharmaceutical manufacturing. *Trends Biotechnol*. 2018;37(3):P253-P267.
2. Klutz S, Magnus J, Lobedann M, et al. Developing the biofacility of the future based on continuous processing and single-use technology. *J Biotechnol*. 2015;213:P120-P130.
3. Konstantinov KB, Cooney CL. White paper on continuous bioprocessing. May 20-21, 2014 Continuous manufacturing symposium. *J Pharm Sci*. 2015;104(3):P813-P820.
4. Walter J, Lu J, Hollenbach M, et al. Perfusion cell culture decreases process and product heterogeneity in a head-to-head comparison with fed-batch. *Biotechnol J*. 2018;14(2).
5. Arnold L, Lee K, Rucker-Pezzini J, Lee JH. Implementation of fully integrated continuous antibody processing: effects on productivity and COGm. *Biotechnol J*. 2019;14(2):P1-P10.
6. Shukla AA, Gottschalk U. Single-use disposable technologies for biopharmaceutical manufacturing. *Trends Biotechnol*. 2013;31(3):P147-P154.
7. Schmidt S. Process intensification in biomanufacturing driven by advances in single use technologies. Single-Use-Technologies III: Scientific and Technological Advancements. Paper presented at: ECI Symposium Series; 2018. http://dc.engconfintl.org/sut_iii/58
8. Heidemann R, Cruz CR, Wu P, Sherman M, Martin J, Fenge C. Single-use bioreactors for the rapid production of preclinical and clinical biopharmaceuticals. *BioPharm Int*. 2014;27(10):P22-P34.
9. Schuirmann DL. On hypothesis testing to determine if the mean of a normal distribution is contained in a known interval. *Biometrics*. 1981;37(3):P617.
10. Westlake WJ, Kirkwood TB. Response to T.B.L. Kirkwood: bioequivalence testing - a need to rethink. *Biometrics*. 1981;37(3):589-594.
11. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2016 R version 3.3.2 (October 31, 2016). http://www.R-project.org/
12. JMP JMP 12.1.0 64-bit edition. SAS Institute Inc. http://www.jmp.com
13. Michaels JD, Mallik AK, Papoutsakis ET. Sparging and agitation-induced injury of cultured animal cells: do cell-to-bubble interactions in the bulk liquid injure cells? *Biotechnol Bioeng*. 1996;51(4):P399-P409.

SUPPORTING INFORMATION

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

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