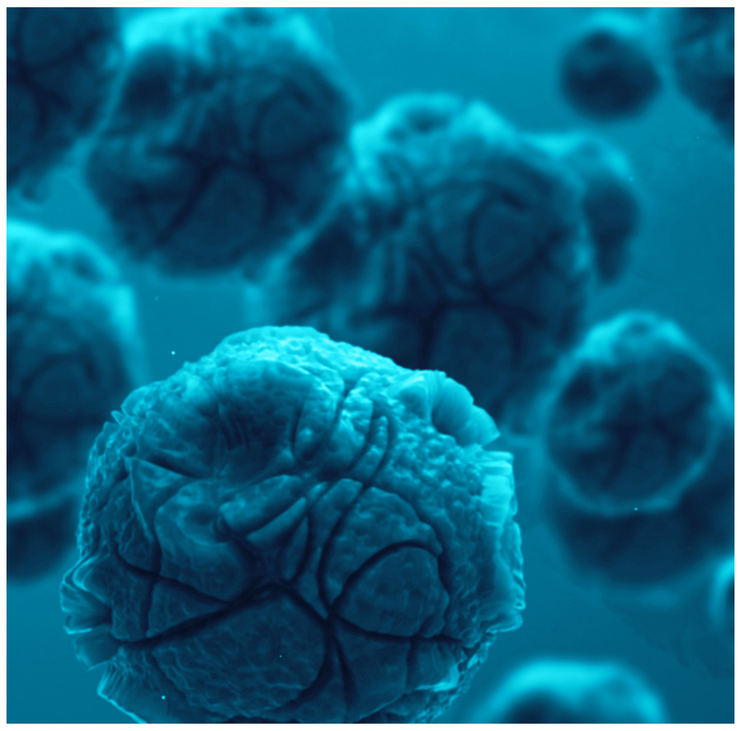
gíbco

Perfusion: best practices for evaluating a perfusion medium

ThermoFisher SCIENTIFIC

Contents



Know your process goal

Evaluating a perfusion process and its associated medium requires numerous factors to be considered. So, how should you start?

Before you begin, you need to understand your process goal and boundaries in terms of acceptable quality and operating limits so that these factors can be approximated in each testing approach. If your product is only acceptable within a certain cell viability limit but a test model doesn't provide controls or plans to actively drive toward that target, the test may be both misleading and less useful for predicting scale-up behavior.

Consider the continuous perfusion operated at two steady states in Figure 1. For the first 19 days, the culture operation is run using a recipe generated from previous studies and achieves an excessively high percent of cell viability. Running with an unnecessarily high cell viability doesn't properly utilize the medium. For example, nutrients are less utilized before they are removed in the spent medium, thus hurting the process efficiency. From day 20 onward, the continuous perfusion run is adjusted to actively target a slightly lower viability at about 95%. This change has a notable impact on the viable cell density (VCD) at steady state and productivity of the process. When the process works at 95% viability, the medium is better utilized by the higher VCD leading to a more efficient process, and achieving a steady-state cell density of about 120 x 10^6 viable cells/mL and a cell-specific productivity (Qp) of about 30 pg/cell/day (approximately 1.7 g/L/day harvest titer).

How a perfusion operation is carried out has significant impact on the results. Clear operating goals and mechanisms to achieve them should be planned out so that the medium tested can be put on a level playing field that is meaningful for the process goals and effectively challenges what is being evaluated.

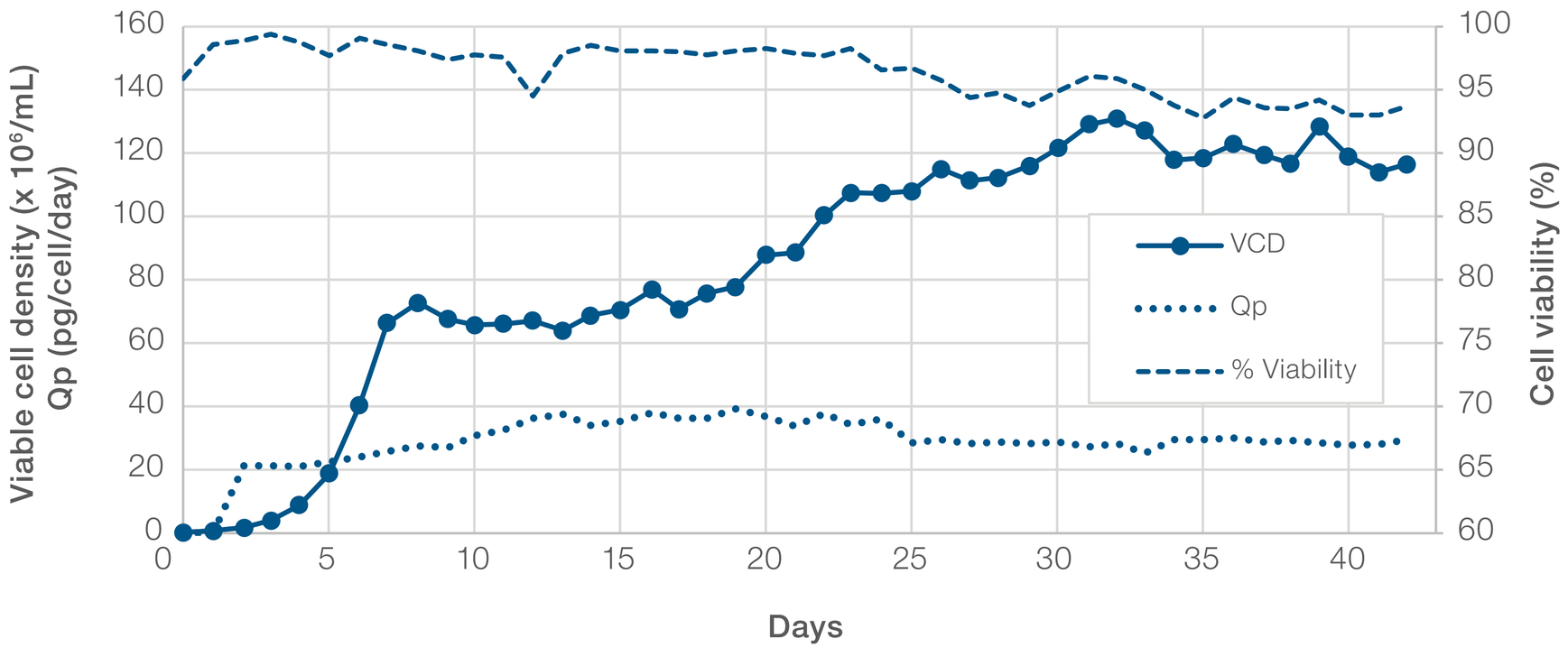


Figure 1. Continuous perfusion at steady state. 1 vessel volume per day (VVD) continuous perfusion using Gibco™ High-Intensity Perfusion (HIP) CHO Medium. At day 19, the operation was changed to better utilize the medium, resulting in a new steady state targeting a lower percent cell viability. New targets were reached around day 26.

Know your test model

TOC

Once clear design goals are established, the next step is to define a test setup. You want the testing to give you results that are meaningful to your process goals and make sure the equipment and materials used don't become accidental limiting factors. The following questions need to be considered before you run your test model:

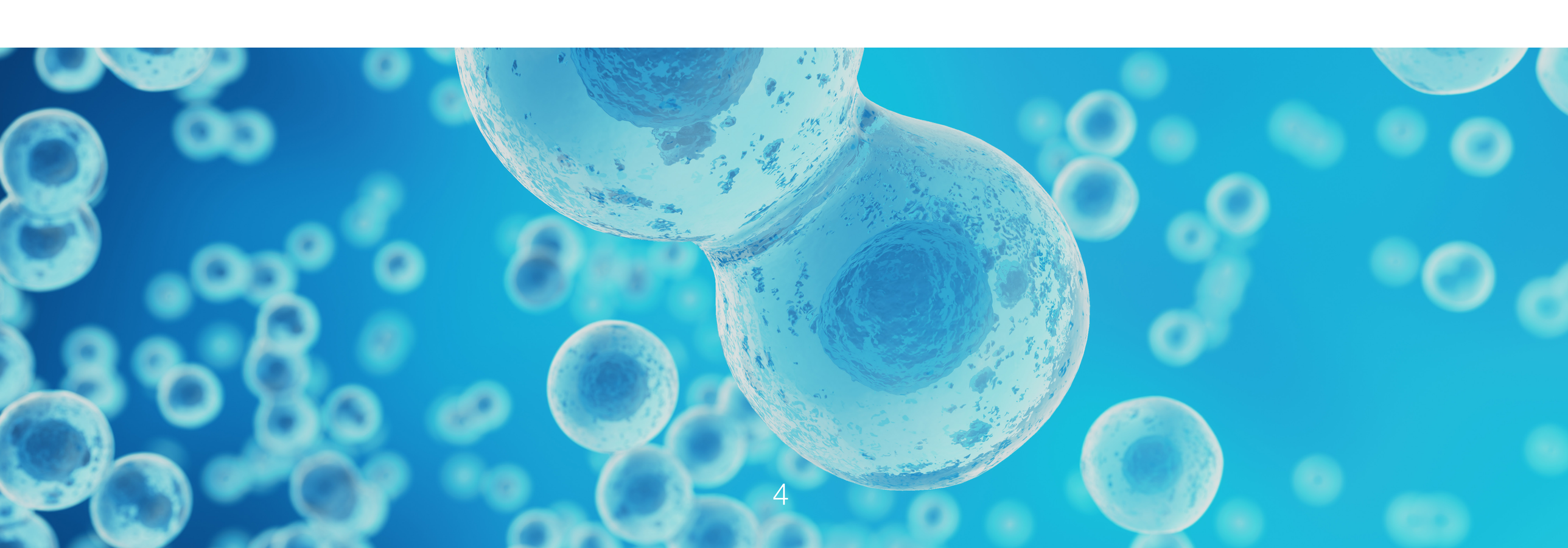
- How long will you run your process?
- How much oxygen transfer can you support?
- What is your target scale-up and what additional restrictions will this cause, such as how much shear will be generated?

Ideally, your test setup allows the systematic evaluation of critical parameters to optimize cell viability and productivity. And, it will help identify the parameters that require scaling and how much flexibility exists. For example, there are multiple approaches to scaling agitation (Table 1), so it is important to know what your process limits are. When combined with your product quality needs, these limits should establish control boundaries for your run, which are meaningful with respect to how you plan to operate the process. This will also help avoid your results being limited by an unplanned, or worse unnoticed, factor in your test model.

Additionally, it will help reduce risk when scaling up the process.

Table 1. Common agitation scaling parameters in a 3 L bench-scale stirred-tank reactor at increasing rpm. Typical animal suspension cell culture agitation power (W/m^3) is highlighted in blue. Progressive comparative minimum tip speeds are highlighted in orange and red with process scale up to 2,000 L, and Kolmogorov eddy length common targets using microcarrier beads are highlighted in green.

Agitation scaling parameters				
Reactor speed (rpm)	**Agitation power (W/m^3)**	**Tip speed (m/sec)**	**Pow num**	**Kolmogorov length (µm)**
55	0.42	0.16	1.99	91.345
75	1.02	0.22	1.89	73.326
200	14.99	0.59	1.47	37.417
239	25.26	0.70	1.45	32.841
292	45.07	0.86	1.42	28.416
348	75.22	1.02	1.40	25.001
389	105.06	1.14	1.40	22.997
443	155.17	1.30	1.40	20.861
486	204.88	1.43	1.40	19.461
555	305.11	1.63	1.40	17.617
610	405.11	1.79	1.40	16.411



Know your cell line

TOC

It is important to know the limits of your cell line. Some of this can be built into your screening test model as noted previously. Some limitations need to be evaluated and considered directly. For example, some cell clones lose productivity too quickly when selective pressure is removed to be able to support a long-term continuous perfusion process. Some cell clones do not behave well at higher VCD, which may limit perfusion benefits. Figure 2 is from a cell line stress run with ramping agitation up to 600 rpm. The cell line handled the stress run and other mechanical stress tests well, but the production stability made it a poor candidate for continuous perfusion tests.

Another limitation to cell line development could be the selection of the medium it was developed in. Before you begin your test, make sure your cell line is fully adapted to any medium conditions. Keep in mind that some cell lines require complex or specialty components from the medium they were developed in. Make sure these are present in all medium conditions tested. Failure to include special components such as growth factors, peptones, and hydrolysates that a cell clone has been developed in can compromise the cell performance and make for a very difficult adaptation. If it is important to remove a specialty component, weening it off should be considered separately after adapting to a new medium with the component present.

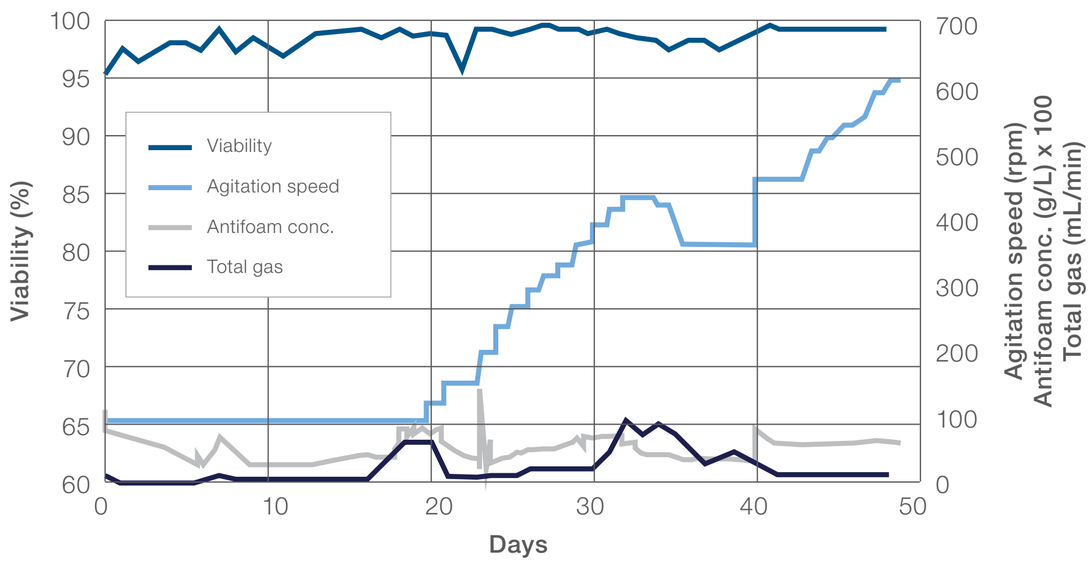


Figure 2. Chemostat operation of a CHO cell culture evaluating varying mechanical stress factors while monitoring cell health.

When adapting your cell line, make sure your growth rate and productivity are stable for two additional passages after the cells seem to have fully recovered. If a cell line adapts readily via direct adaptation, you should still run a total of six adaptation passages to ensure it is behaving well in the new medium with little-to-no carryover from the previous formulation.

Also remember, when operating in perfusion, medium is constantly exchanged, so supplements must be considered carefully. Some supplements that normally accumulate in fed-batch culture will be continuously removed in perfusion and may need concentrations increased. Some supplements that are once dosed early, such as L-glutamine, will be constantly added back in perfusion and may benefit from reduced concentration. And finally, some components accumulate in the cells themselves and should be supplemented with caution to avoid overloading the cells over time.

Know your assumptions

TOC

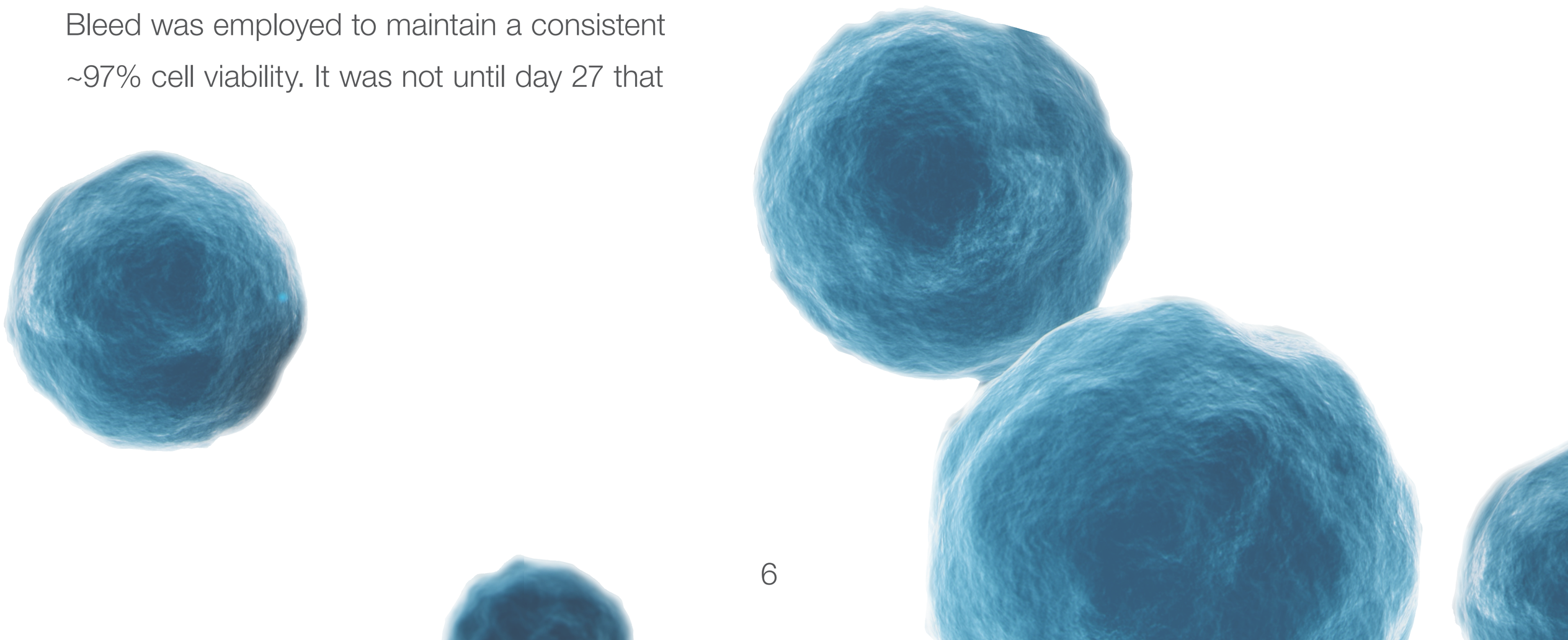
It is equally important to understand what you are not testing as it is to know what you are testing in your model. It is generally not practical to cover all critical process parameters in a screening model. However, anything not covered in a screening model is a risk that should be evaluated independently, or it may catch you by surprise later. For example, if you have already demonstrated that your cell clone is mechanically highly robust, it may not be important to get agitation scaling and other mechanical stress factors right in your screening model. Having a clear understanding of what risks mean for your cell clone and process goals is critical when making assumptions and conclusions about generated data. Bolus vs. continuous fluid handling, using different mechanisms to retain cells, weak scaling representation, and other operating differences should all be evaluated.

For example, one of the biggest weaknesses in most screening models is time. Typical screening tests only run for about 7–15 days. This may not be enough to see limits and final behavior of a given process and medium. In Figure 3, a continuous 1 VVD perfusion run was carried out in triplicate. Bleed was employed to maintain a consistent ~97% cell viability. It was not until day 27 that this process approached a final steady state. Had it been screened in the first 10 to 15 days, the conclusions would not have given a realistic representation of the cell line and would suggest a steady state limit of ~1 g/L harvest titer, as opposed to what seems to be the cell line and medium's ultimate final behavior in a continuous process of around 1.6 g/L harvest titer.

By comparison, other media tested have demonstrated the opposite behavior—showing high early productivity on day 0 through 10 but reduced productivity over time at a faster rate of decay than what the native cell line's productivity decline looked like during passaging without selective pressure.

Some cell lines exhibit long periods of metabolic oscillations that require additional efforts to mitigate.

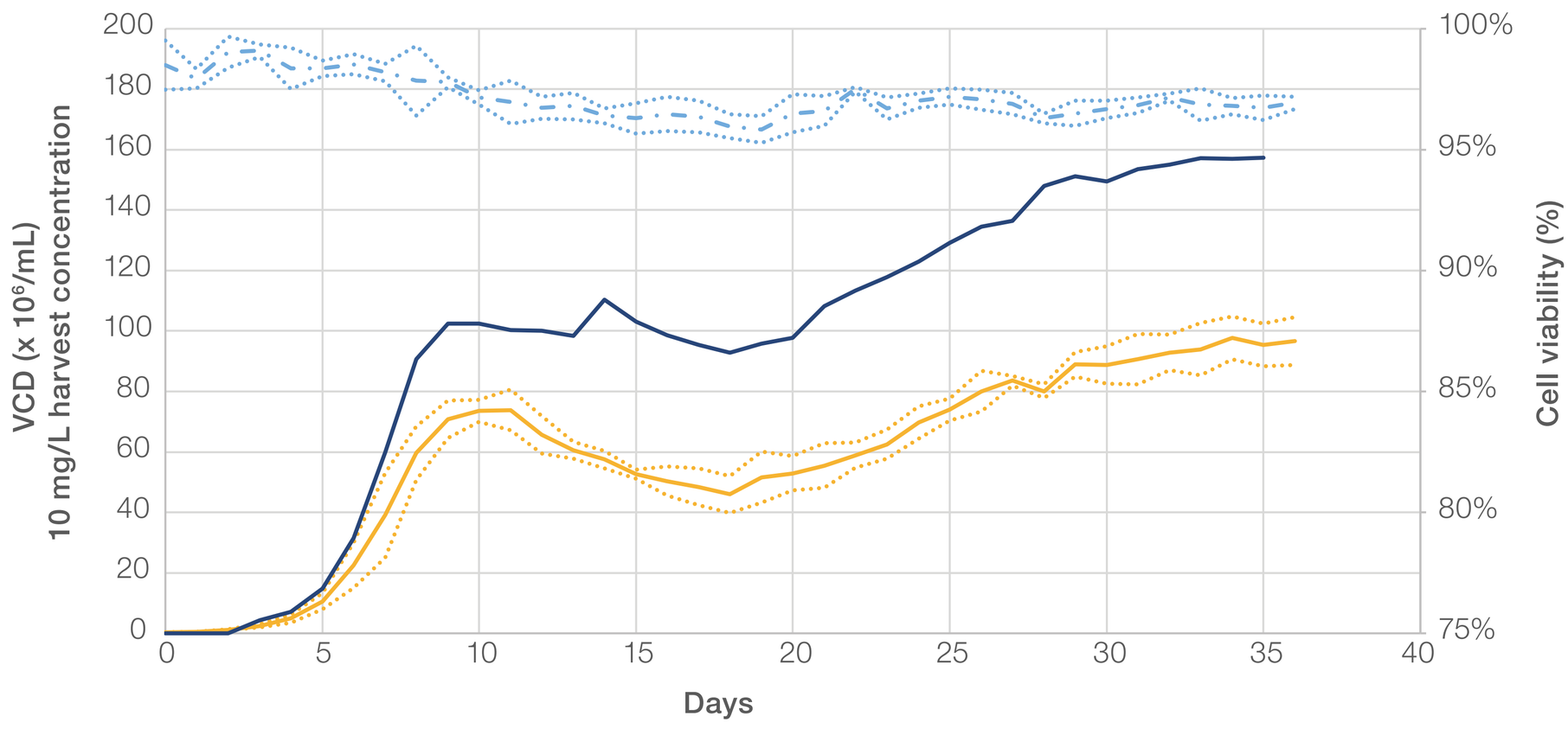
On an even simpler level, operating perfusion screening for too short of a duration in some cases doesn't allow enough time for cells to start dying, leaving a misleading impression about what VCD can be sustained at a given target percent cell viability.



Ultimately, it is important to benchmark any screening process with an actual perfusion run to help understand which performance assumptions are reasonable and which risks are practical.

When evaluating a perfusion process and the medium to be used, keep the following key points in mind:

- Have a clear goal and design space determined for your screening so that you can build on top of your data and be confident in your comparisons.
- Make sure your test model and equipment are robust enough to deliver the performance you are looking for within your design space and don’t accidentally limit behaviors.
- Understand your cell line’s needs prior to developing a screening model and make sure the formulation comparisons are complete before adapting the model.
- Know and account for the limits in what your screening model doesn’t address and plan for validation runs to help ensure your assumptions are reasonable.

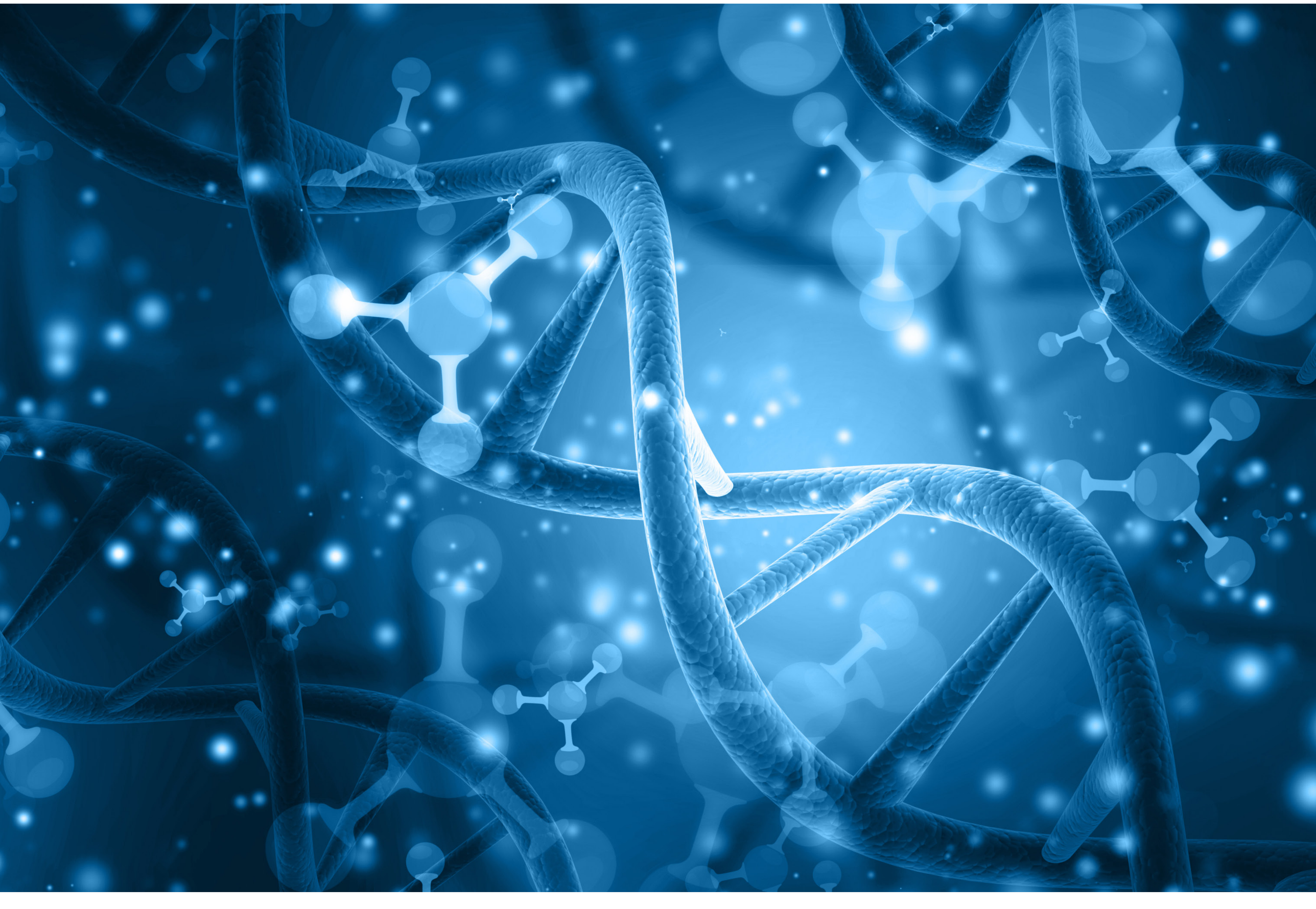


Figures 3. Continous perfusion operation at 1 VVD targeting constant cell viability, run in triplicate.
Dotted lines are ±1 standard deviation. Note: harvest titer is in units of 10 mg/L.

gíbco

Additional resources

For additional resources, go to **thermofisher.com/perfusion**.



Find out more at **thermofisher.com/perfusion**

ThermoFisher SCIENTIFIC