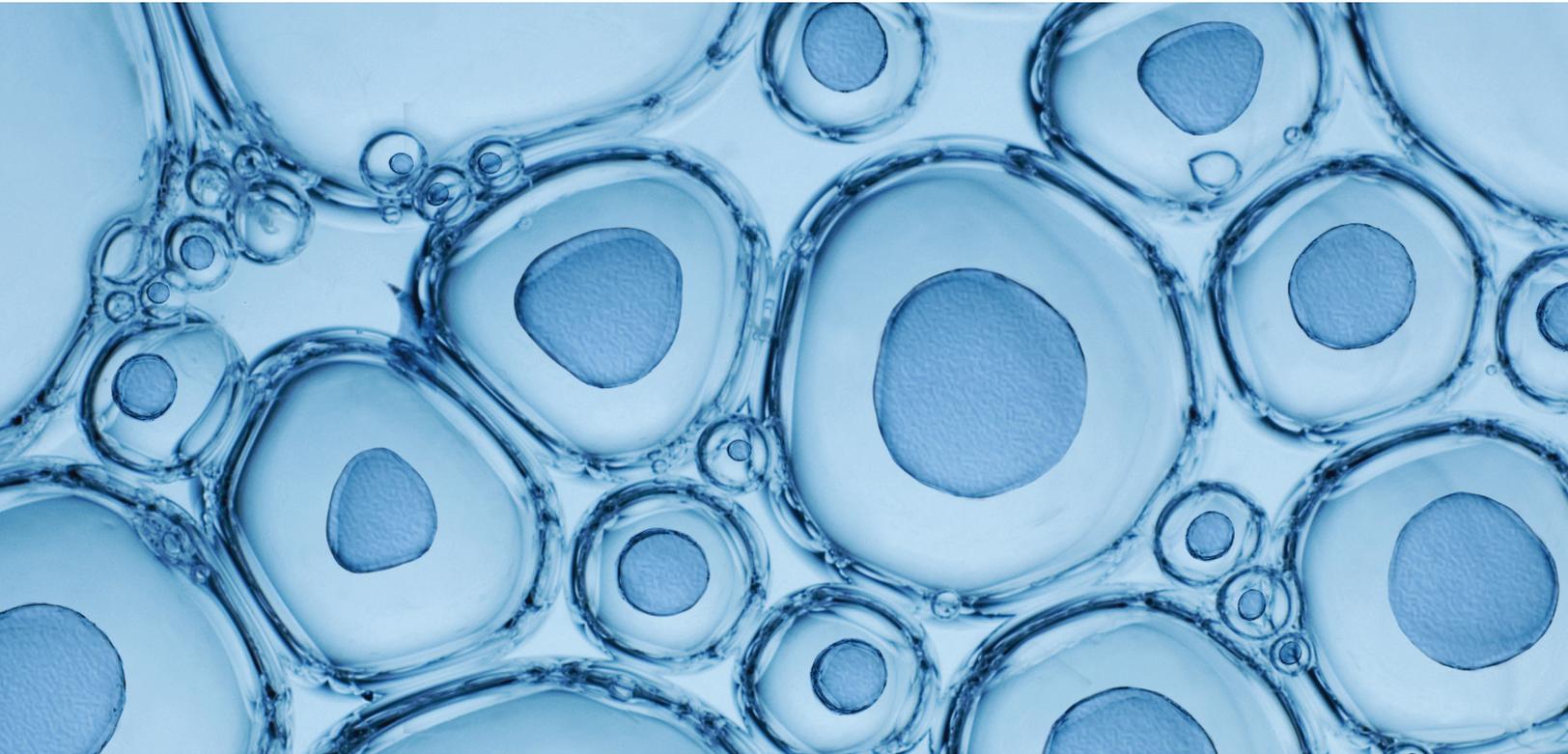


Performance challenging Fetal Bovine Serum (FBS) and FBS alternatives

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ABSTRACT

Growth performance testing in cell culture is an effective approach to making serum suitability and purchase decisions.¹ An independent commercial testing lab conducted two separate and sequential growth promotion studies to underscore the need for pre-purchase lot performance testing with: (1) FBS, and (2) FBS alternatives.

Results from both studies are presented here to compare and contrast:

- FBS lots to each other
- FBS alternatives lots to each other
- FBS alternatives lots to FBS

FBS alternatives are included because they are often overlooked as a cost-effective substitute for FBS, providing, in many cases, equivalent performance.

It is advisable to avoid preconceived notions concerning the quality or performance of a serum product without considering the culture system, culture conditions, and the subject cells, which can all have a material impact on its performance in cell culture. Test – then decide.

1. FBS PERFORMANCE CHALLENGE

Test article (TA) lot performance was evaluated with three different cell lines at four TA concentrations. Additionally, the impact of TA concentration used in the cell culture medium was examined.

FBS end-users employ various methods for assessing the quality of FBS in cell culture. One of the most effective approaches is low-density plating efficiency testing.^{1,2} Plating efficiency is an individual characteristic of each adherent cell line (adhered/plated × 100). At clonal seeding density, the cells are unable to adequately condition the medium and must depend

heavily on the nutritional capacity of the culture medium. The experimental design used in these two plating efficiency studies is based upon protocols cited above.^{1,2}

1.1 Materials and Methods

Plating efficiency performance testing was conducted by an independent commercial testing lab on randomly selected production lots of FBS from three vendors (VWR Life Science Seradigm, GE HyClone, and ThermoFisher Gibco) with two lots from each vendor (Table 1). FBS lots were tested in triplicate at four concentrations: 10%, 7.5%, 5.0%, and 2.5%. The FBS control was the test lab's in-house lot to which all three cell lines were adapted pre-assay. The FBS control lot was not identical to any of the TA lots.

Additionally, one lot of VWR Life Science Seradigm FB Essence, an FBS alternative, was included for comparison with this FBS performance challenge.

Three adherent-dependent cell types were obtained from ATCC: CCL-34 (MDCK), CCL-75 (WI-38), and CCL-61 (CHO-K1). MDCK and CHO-K1 are cell lines that can be passaged indefinitely. WI-38 is a well-characterized normal human cell with a finite lifespan of approximately 50 population doublings (not passages). All three cell lines have been used in vaccine production. Clonal cell-seeding levels are different for each cell line and were determined for this study by preliminary testing. Three cell-seeding levels were examined for each cell line to ensure that usable data was generated. The seeding level delivering the most information for the most test articles was selected for analysis.

Culture medium consisted of fortified Eagle's minimum essential medium (EMEM) with the FBS or FBS alternative TA. The EMEM (without L-glutamine) containing Earle's salts was enriched with 0.1mM non-essential amino acids (1×NEAA) + 1mM sodium pyruvate + 2mM L-alanyl-L-glutamine (stable glutamine). The NEAA is included to support CHO-K1 cells that require L-proline, which is not included in EMEM. Sodium pyruvate improves plating

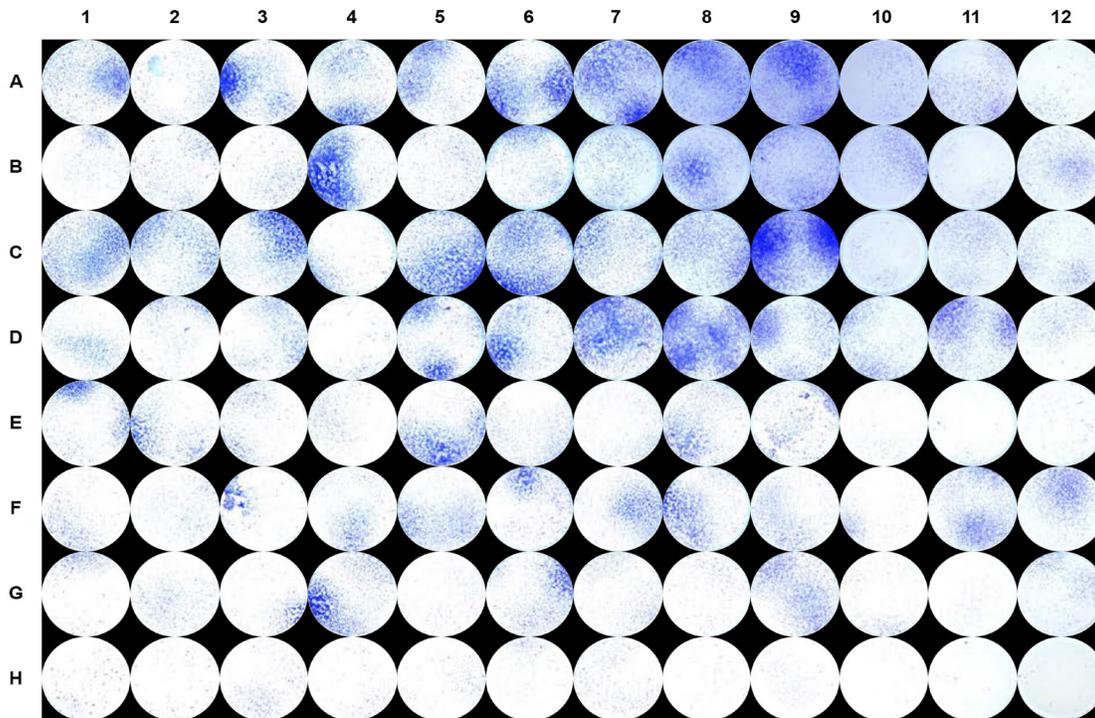


FIGURE 1. FBS performance challenge. Plate image of MDCK cells: TAs 1–8 are displayed in order, top to bottom, in rows A–H. TA concentrations: 10%, columns 1–3; 7.5%, columns 4–6; 5.0%, columns 7–9; and 2.5%, columns 10–12.

efficiencies.³ The stable form of L-glutamine eliminates concerns about toxic accumulation of ammonium ions, a natural product of L-glutamine decomposition in media.

Ammonium ion toxicity is not a concern in culture conditions that have regular feeding schedules with medium replacement and higher cell-seeding densities. There was no feeding or culture fluid replacement during the ten-day incubations.

All cell lines were adapted to the culture plating medium plus 10% control FBS for several passages before initiation of testing. Thus, only one nutritional component was changed at the onset of testing, the TA.

Cells were seeded into 96-well Eppendorf plates. MDCK was seeded at 200, 100, and 50 cells per well. WI-38 was seeded at 320, 160, and 80 cells per well. CHO-K1 was seeded at 80, 40, and 20 cells per well. To prevent evaporation edge effects, the moat surrounding the wells was filled with Dulbecco's phosphate-buffered saline (DPBS).

The plates were incubated undisturbed and unfed for ten days at 37°C with 5–7% CO₂, then fixed in 1:1 DPBS/methanol and stained with 0.5% crystal violet.

Stained plates were evaluated with an optical imaging CTL S6 Macro analyzer (ImmunoSpot). This equipment is capable of determining colony spot counts and area-percent confluency. Cell morphology in the plates determined whether spot counts or area-percent confluency was used. Area-percent confluency was used for evaluating the results of the first study. Separately, spot count data was used with one result in the second study (below). Triplicate well data was averaged and plotted graphically.

Objective instrumental evaluation eliminates the potential for biased interpretation of results. Higher performance is defined as more colony spot counts or a larger area-percent covered with cells (Figure 1). In other words, this is evidence for better nutritional support from the TA.

TA #	Supplier	Description	Catalog #	Lot #
1	VWR Seradigm	FBS	89510-194	013B16
2	VWR Seradigm	FBS	89510-194	074B17
3	Testing Lab	Control FBS	—	—
4	VWR Seradigm	FB Essence	10805-184	048B16
5	GE Hyclone	FBS	SH30071.02	AB10104797
6	GE Hyclone	FBS	SH30071.02	AB10095803
7	ThermoFisher Gibco	FBS	A3160501	1883391
8	ThermoFisher Gibco	FBS	A3160501	1876851

TABLE 1. Test article key, FBS performance challenge.

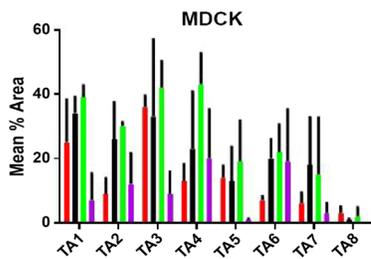


FIGURE 2. FBS performance challenge. Plate graph of the MDCK cells shown in Figure 1.

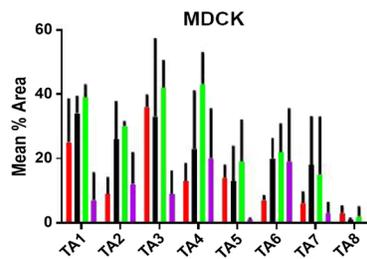


FIGURE 3. FBS performance challenge. Plate graph of WI-38 cells.

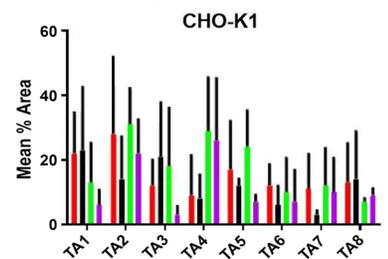


FIGURE 4. FBS performance challenge. Plate graph of CHO-K1 cells.

1.2 Results

VWR Life Science Seradigm FBS:

- Provided consistently superior nutritional support.
- Both lots of VWR Life Science Seradigm FBS outperformed both lots of GE HyClone and both lots of ThermoFisher Gibco on all three cell lines tested (Figures 2,3,4).
- Both lots of VWR Life Science Seradigm FBS performed equivalently to Seradigm FB Essence (Figures 2, 4).

VWR Life Science Seradigm FB Essence (an FBS alternative):

- Equaled or exceeded FBS performance on MDCK and CHO-K1 cells, as compared to both lots of GE HyClone and both lots of ThermoFisher Gibco (Figures 2,4).

2. FBS ALTERNATIVES PERFORMANCE CHALLENGE

FBS is subject to dramatic price swings that are coupled to wide fluctuations in supply. FBS alternatives are suitable for many applications, and are commercially offered with three main benefits to the end-user:

- Increased savings as a cost-effective substitute for FBS.
- Enhanced supply chain continuity.
- Improved consistency in cell culture performance from lot-to-lot because of manufacturers' proprietary supplementations.

2.1 Materials and Methods

Randomly selected production lots of FBS alternatives were as follows: VWR Life Science Seradigm FB Essence, Gemini FetalPlex,

TA #	Supplier	Description	Catalog #	Lot #
1	VWR Seradigm	FB Essence	10805-184	048B16
2	VWR Seradigm	FBS Essence	10805-184	203B15
3	VWR Seradigm	FB Essence	10805-184	203B15
4	Gemini	FetalPlex	100-602	J77J001
5	Atlas	EquaFETAL	SH30071.02	AB10104797
6	Testing Lab	Control FBS	—	—
7	GE Hyclone	Fetal Clone II	SH30066.2	AB217603
8	GE Hyclone	Fetal Clone III	SH30066.2	AB217603

TABLE 2. Test article key, FBS alternatives performance challenge.

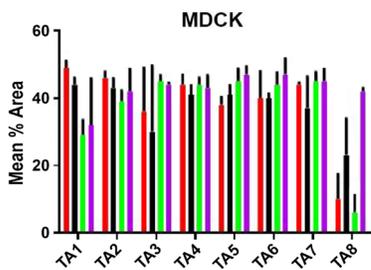


FIGURE 5. FBS alternatives performance challenge. VWR FB Essence equaled or exceeded other FBS alternatives. HyClone FetalClone III (TA8, optimized for broad applications) performance was notably less for MDCK cells, as compared to other samples, except at the most dilute concentration. This outcome did not reappear in the TA8 results with WI-38 and CHO-K1 cells (Figures 6, 7).

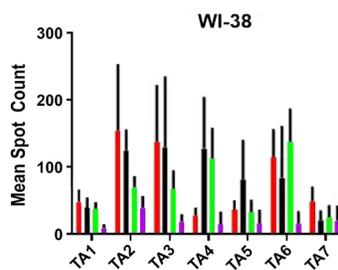


FIGURE 6. FBS alternatives performance challenge. VWR FB Essence equaled or exceeded the FBS control (TA6) for two of three VWR lots.

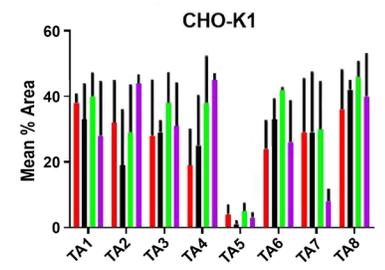


FIGURE 7. FBS alternatives performance challenge. VWR equaled or exceeded other FBS alternatives. With TA5, Atlas EquaFETAL, performance was notably less for CHO-K1 cells than for MDCK or WI-38.

Atlas EquaFETAL, HyClone FetalClone II (optimized for CHO-K1), and HyClone FetalClone III (optimized for broad application) (Table 2). HyClone FetalClone I was not included because it is optimized for hybridomas, which are typically adherent-independent suspension cultures. All cell lines employed were adherent-dependent.

The plating efficiency performance evaluation was conducted as described in section 1. The same stocks of cells adapted to the same EMEM culture medium plus 10% FBS control were used. The only nutritional component changed for the study was the FBS alternative supplement, which was substituted for the FBS control. Colony spot counts were used for WI-38 results evaluation because it correlated well with the cell morphology in these results.

2.2 Results

VWR Life Science Seradigm FB Essence performance equaled or exceeded that of FBS and other FBS alternatives.

FB Essence performance vs. FBS:

- Equaled two lots of FBS from VWR Life Science Seradigm (Figures 2, 4).
- Matched the FBS control (all three lots of FB Essence on all three cell lines) except for TA1 on WI-38 (Figures 5, 6, 7).
- Exceeded FBS lots from GE HyClone and ThermoFisher Gibco (Figure 2).

FB Essence performance vs. other FBS alternatives:

- Equaled or exceeded other FBS alternatives on test (Figures 5, 6, 7).

3. OVERALL STUDY RESULTS

3.1 Impact of FBS and FBS Alternatives Concentrations

Serum supplementation at 10% has been the accepted standard for many years. This FBS and FBS alternatives study revealed that in many cases, plating efficiency performance was improved at 5% supplementation. This suggests that, at clonal plating densities, slight inhibitory effects can be overcome with dilution, both for FBS alternatives and for FBS (Figures 2,7).

In Figure 2 with FBS performance challenge and MDCK cells, performance at 5% concentration exceeded that at 10% for seven of eight TAs, including the control FBS and the FBS alternative, FB Essence. Figure 7 exhibits a similar pattern with FBS alternatives performance challenge and CHO-K1 cells. Here again, seven of eight TAs displayed better performance at 5% than at 10%, including the control FBS. However, the performance edge was slight in two of these TAs, 5 and 7.

4. DISCUSSION

4.1 FBS and FBS Alternatives Performance Challenge

FBS performance is variable from lot-to-lot and depends on the specific application. Variability can be attributed to two main sources: natural biological variation and manufacturing.

Some performance inconsistency is inherent in the biological variation of a natural product, over which manufacturers can exert no control. Conversely, to preserve native performance potential, manufacturers can exert quality controls over every facet of converting raw material into finished goods.

REFERENCES

1. Freshney RI. Section 8.6.2, Testing serum. In Culture of animal cells: a manual of basic technique and specialized applications, 7th edition. John Wiley & Sons, Inc., Hoboken, New Jersey; 2016.
2. Hay RJ, Caputo J, Macy ML, eds. ATCC quality control methods for cell lines, 2nd edition. American Type Culture Collection, Rockville, Maryland; 1992; pp 7-10.



Performance potential can be reduced during collection, handling, and processing of raw material into finished goods. End-users should consider sourcing from manufacturers that use methods and technology to retain maximal performance and minimize avoidable losses in quality.

End-users should strongly consider FBS alternatives as one approach to reducing lot-to-lot variation. Additional reasons for the customer to do so are cost savings, better purchase planning and management, more predictable availability and pricing, and in some cases, equivalent or even superior performance to FBS in cell culture.

Additionally, as part of pre-purchase performance testing, end-users may be able to identify further cost savings by reducing the serum concentration. Experiment with your culture system to determine if this is a valid approach for your applications.

Manufacturers strive to attain finished goods of high quality and consistent performance. These studies highlight the nutritional performance of FBS and FBS alternatives and show that cell lines may not respond with similar robustness under similar conditions. It demonstrates the need for pre-purchase lot testing, especially for cell lines with fastidious nutritional requirements.

3. Freshney RI. Section 16.2.1, Conditions that improve clonal growth. In Culture of animal cells: a manual of basic technique and specialized applications, 7th edition. John Wiley & Sons, Inc., Hoboken, New Jersey; 2016.