

The Effects of FBS Storage Temperature on Cell Viability and Growth

Background

Foetal bovine serum (FBS) is an essential component of cell culture media, providing vital growth factors, hormones, and nutrients to support cell proliferation. Typically, FBS is stored at -20°C to maintain its quality and ensure consistent cell growth and viability in research and production environments. However, certain laboratory conditions may require FBS to be stored at 4°C, raising questions about the potential impact on cell culture outcomes. This study investigates the effect of storing FBS at 4°C compared to the standard -20°C on the viability and growth of several key cell lines.

Study Overview

This analysis examined the effects of FBS storage at -20°C and 4°C on three different batches of serum across four widely used cell lines: MDCK, L929, C2C12, and Sp2O/Ag14. Cell growth and viability were evaluated in various conditions, including low seeding densities and different sub-cultures, to determine how storage temperature affects performance.

Key Findings

- C2C12 (Mouse Myoblast Cells)**
 - Growth:** FBS stored at 4°C resulted in significantly reduced growth for C2C12 cells. This reduction was particularly evident under low seeding conditions, where growth decreased by 39.6% compared to cells cultured with FBS stored at -20°C. Overall, C2C12 cells demonstrated better growth when FBS was stored at -20°C across all conditions.
 - Viability:** Although the drop in viability was small, it was notable that C2C12 cells experienced a decrease from 97.5% with FBS stored at -20°C to 96.3% with FBS stored at 4°C. This suggests some sensitivity of this cell line to storage temperature, even if the impact on viability is relatively modest.

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The Science Centre, 1 Blaydon Road, Sandy, Bedfordshire, SG19 1RZ

Tel: +44 (0) 1234 889180 Email: sales@lifesciencegroup.co.uk Web: www.lifesciencegroup.co.uk

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2. L929 (Mouse Fibroblast Cells)

- **Growth:** L929 cells showed a moderate decline in growth when FBS was stored at 4°C, particularly in Sub-culture 1 and low seeding conditions. However, the overall growth reduction was less severe compared to C2C12 cells, suggesting L929 cells are more resilient to changes in FBS storage conditions.
- **Viability:** Importantly, L929 cells maintained high viability levels (>95%) regardless of the FBS storage temperature, making them robust and suitable for use in conditions where storage flexibility is needed.

3. MDCK (Madin-Darby Canine Kidney Cells)

- **Growth:** MDCK cells exhibited minimal differences in growth between the two storage conditions in Sub-culture 1. Interestingly, these cells showed improved growth under low seeding conditions when FBS was stored at 4°C, suggesting that for certain experimental conditions, FBS stored at higher temperatures may be advantageous.
- **Viability:** While there was a slight decrease in viability at 4°C, particularly in Sub-culture 1, MDCK cells performed well under low seeding conditions with FBS stored at 4°C, achieving viability rates of 98.83%. This indicates that MDCK cells may tolerate or even benefit from FBS stored at 4°C in specific contexts.

4. Sp2O/Ag14 (Mouse Hybridoma Cells)

- **Growth:** Sp2O/Ag14 cells consistently exhibited reduced growth when cultured with FBS stored at 4°C. This was especially pronounced in low seeding conditions, where growth significantly declined.
- **Viability:** The viability of Sp2O/Ag14 cells was notably impacted by FBS storage temperature. Viability dropped to 66.87% in Sub-culture 2 and 69.27% in low seeding conditions when FBS was stored at 4°C, highlighting the sensitivity of this cell line to suboptimal storage conditions.

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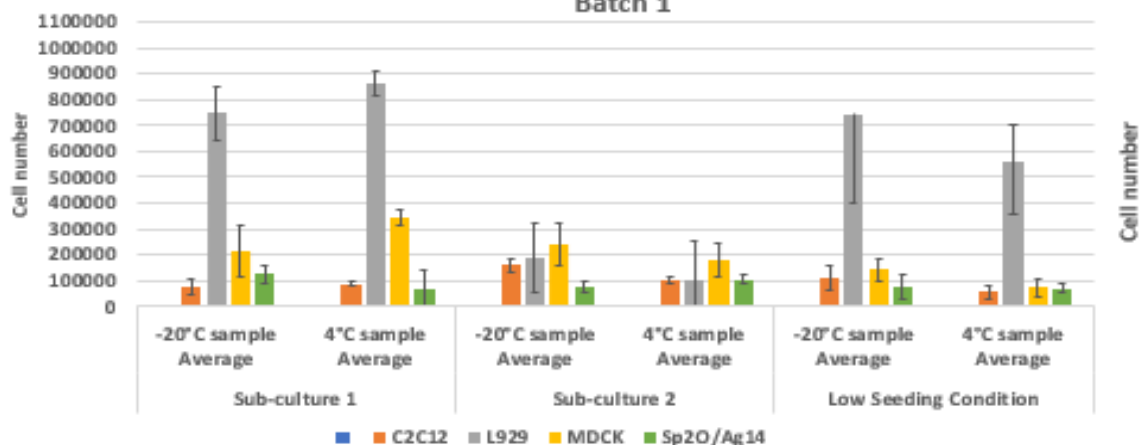
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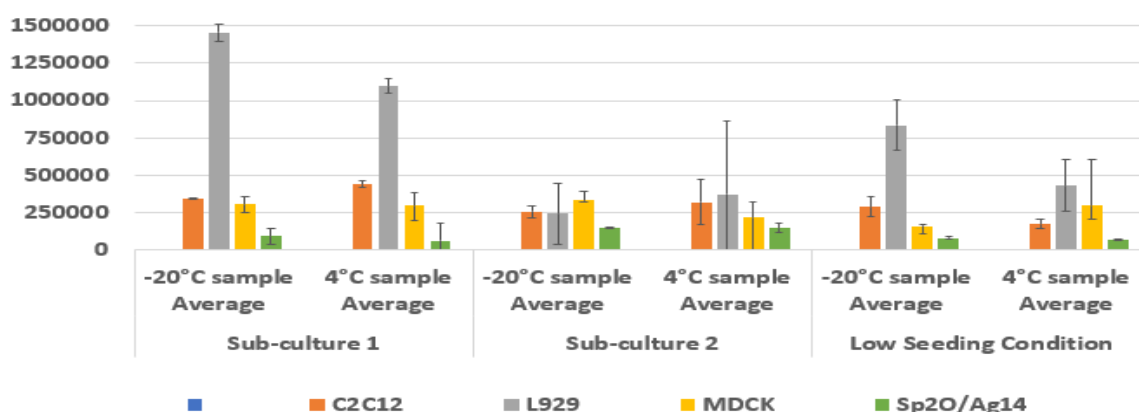
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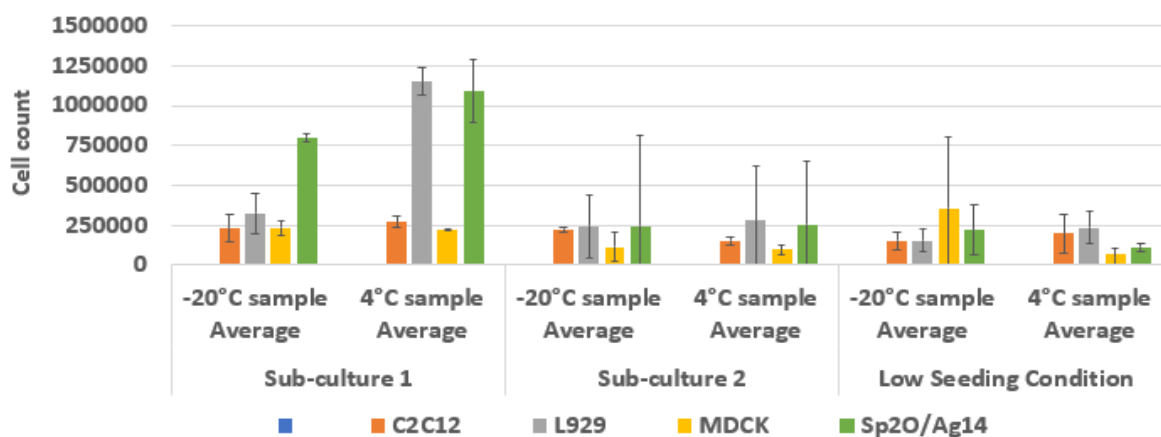
Effects of using FBS stored in different temperatures on cell growth
Batch 1



Effects of using FBS stored in different temperatures on cell growth
Batch 2



Effects of using FBS stored in different temperatures on cell growth
Batch 3



Conclusions

The study underscores the critical role FBS storage temperature plays in supporting optimal cell viability and growth. The findings provide important guidelines for laboratories handling FBS across different cell lines:

1. **C2C12 and Sp2O/Ag14 Cells:** These cell lines exhibit significant reductions in both growth and viability when FBS is stored at 4°C, particularly in low seeding conditions. Therefore, storing FBS at -20°C is strongly recommended for these cells to ensure optimal culture performance.
2. **L929 Cells:** L929 cells are highly resilient, showing only moderate growth reductions and maintaining high viability levels at both storage temperatures. This flexibility allows labs to store FBS at either -20°C or 4°C when culturing L929 cells, depending on other logistical constraints.
3. **MDCK Cells:** MDCK cells demonstrated minimal negative impact from FBS stored at 4°C and even showed improved growth in low seeding conditions. This suggests that MDCK cells are well-suited to conditions where FBS must be stored at higher temperatures, making them ideal for experiments that require storage flexibility.

Practical Implications

For laboratories working with multiple cell lines, these findings highlight the importance of tailoring FBS storage practices to the specific requirements of each cell line. While some cell types, like L929 and MDCK, can tolerate or even benefit from FBS stored at 4°C, others—such as C2C12 and Sp2O/Ag14—require stricter storage conditions to sustain viability and growth.

Optimising FBS storage temperatures based on the cell line's needs can significantly improve experimental outcomes, ensuring consistent, reliable results in both research and production environments. For sensitive cell lines, maintaining FBS at -20°C is crucial, while for more robust lines, such as MDCK and L929, storage flexibility can be considered without compromising performance.

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Conclusion

In summary, the effects of FBS storage temperature vary considerably across different cell lines. This study provides clear evidence that certain cell types, particularly C2C12 and Sp2O/Ag14, require FBS to be stored at -20°C for optimal viability and growth. On the other hand, more resilient lines like L929 and MDCK offer flexibility, allowing laboratories to adapt their storage practices without significantly affecting culture outcomes. Understanding these variations enables researchers to make informed decisions about FBS handling and storage, improving the efficiency and effectiveness of cell culture protocols.

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