**B8 Production of monoclonal antibodies in suspension culture of D1-4G2-4-15 hybridoma using spinner flasks system**

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**Introduction:** Production of monoclonal antibodies involves *in vivo* or *in vitro* procedures. *In vivo* production presents great variability and difficulty of scaling-up, as well as ethical problems. *In vitro* processes are robust and achieve high cell densities, an easy scaling-up, and a better control/regulation of the process. The most widely used *in vitro* systems for antibody production include *spinners* and *rollers* flasks and bioreactors. Culture conditions have a significant impact on the cell growth and antibody production. In this work, D1-4G2-4-15 hybridoma, producer of monoclonal antibodies anti-flavivirus group antigen, were cultivated. Cellular viability, cell growth rate, IgG production, glucose and L-lactate concentrations have been used as investigation criteria in order to establish the optimized conditions of antibody production in spinner flasks.

**Objective:** Establish a suspension culture in spinner-flask of D1-4G2-4-15 hybridoma.

**Methodology:** D1-4G2-4-15 murine hybridoma was grown in suspension using both pendulum and impeller *spinners* in high glucose medium supplemented with fetal bovine serum and LGlutamine. Initially, the hybridoma culture was adapted to growth in suspension, maintaining the culture at low cellular concentration and changing the medium periodically to keep it in exponential growth phase. Subsequently, cell growth and IgG production kinetic of hybridoma were carried out to characterize the suspension culture in batch mode. Finally, the suspension culture was carried out in semi-continuous mode changing medium every 48h to evaluate the cell culture behaviour at three different cell concentration ranges. Samples were taken in order to quantify metabolites and IgG production.

**Results:** The hybridoma adaptation to suspension culture was successfully implemented in both pendulum and impeller *spinners*, with specific growth rate of 0.038±0.004h-1 and 0.031±0.005h-1 respectively, and viability above 90%. The cell growth kinetic in suspension culture presented an exponential cell growth during the first 48h, followed by a 24h stationary phase. The maximum viable cells concentration in pendulum *spinners* was about 25% higher than that obtained in impeller *spinners*. The IgG production reached a maximum of 139±22mg/L (day 5 and 6 - pendulum) and 123±23mg/L (day 5 to 8 - impeller). Based on these results, 10-day culture in semi-continuous mode using pendulum spinners proceeded within the range of 0.4 - 2.0 x106 cel/mL. The average specific growth rate was 0.031±0.03h-1. The

IgG concentration ranged 17±3 - 88±11mg/L. Both results are in agreement with the literature data published. Higher cell concentration range culture at the same conditions was not feasible.

**Conclusion:** The suspension culture of D1-4G2-4-15 hybridoma in semi-continuous mode was established using spinner flasks. Maximum levels of IgG obtained were similar to those reported in the literature for this type of culture. The results are promising and will serve for future studies using serum-free media in order to optimize the production process.

**Keywords:** Hybridoma, Suspension Culture, IgG Production