TESTING FCS BATCHES FOR CULTURE OF EMBRYONIC STEM CELLS

1. Colony assay

- Order different test batches of FCS at the Purchase Department, e.g. 10 different batches.

- Set up complete media + β + LIF in gelatin-coated 6 well multidishes (2 ml/well) with twice the intended final concentration of FCS's, e.g. 10% (by volume), 20% and 40%. Equilibrate in the incubator at 37 °C/5% CO_2 before use. Use duplicate wells for each condition and appropiate controls (e.g. known "good" serum batch).

- Trypsinize an ES cell stock culture on day 2 after plating (i.e. semi confluent), ensuring as close to a single cell suspension as possible. Count and resuspend in complete medium + β + LIF without serum, at a density of 10^3 cells/ml.

- Add 2 ml of cell suspension to each well (making 4 ml total volume 1x concentration of serum) and return to incubator.

- Incubate for 5 days at 37 °C/5% CO_2 in air.

- Stain with Leishman's:
  . wash cells once with PBS.
  . add 1 ml of Leishman's staining solution; 5 min RT.
  . add 7 ml of Gurr's buffer pH 6.8; 5 min RT.
  . wash several times with tap water.

ES cell colonies can be identified by characteristic morphology and dark staining properties. Differentiated colonies are more pale in colour. Count the total number of colonies (=plating index) and the proportion of ES cell colonies. For FCS batch testing the plating efficiency is the relevant parameter (along with the colony size); for LIF testing, the proportion of differentiated colonies is the relevant parameter.

2. Culture assay

- Select the four best FCS batches and the reference batch and start to make conditioned medium with these batches.

- Culture a good batch of ES cells on these conditioned media (60%) on 6-well plates coated with gelatin. Follow the cells for about three passages and judge them on morphology and growth rate.

- It is best to judge the ES cells with more people. Judge them at least every passage, best is every day. Cells can change their behaviour during culture.