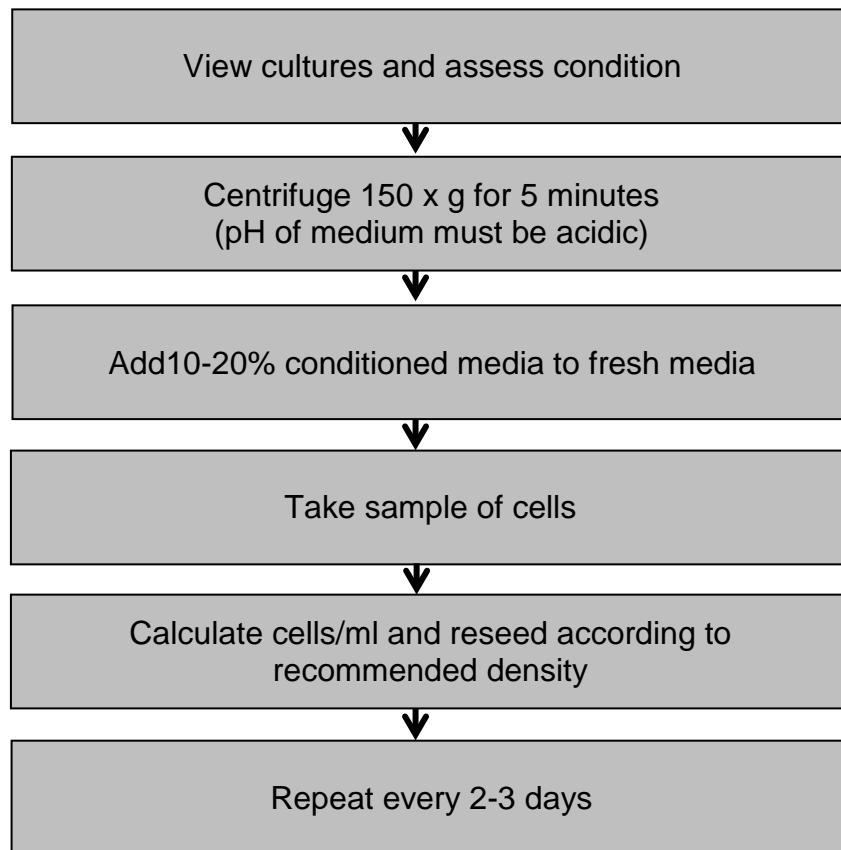


Cell culture protocol:

Subculture of suspension cell lines



Aim

In general terms cultures derived from blood (e.g. lymphocytes) grow in suspension. Cells may grow as single cells or in clumps (e.g. EBV transformed lymphoblastoid cell lines). For these types of cell lines subculture by dilution is relatively easy. However, for cell lines that grow in clumps it may be necessary to bring the cells into a single cell suspension by centrifugation and resuspension by pipetting in a smaller volume before counting.

Materials

- media – pre-warmed to 37°C (refer to the ECACC Cell Line Data Sheet for the correct medium)
- 70% (v/v) isopropanol in sterile water
- Trypan blue (vital stain)

Cell culture protocol:

Subculture of suspension cell lines

Equipment

- personal protective equipment (sterile gloves, laboratory coat, safety visor)
- water bath set to 37°C
- microbiological safety cabinet at appropriate containment level
- centrifuge
- incubator
- inverted phase contrast microscope
- haemocytometer
- pre-labelled flasks
- marker pen
- pipettes

Procedure

1. View cultures using an inverted phase contrast microscope. Cells growing in exponential growth phase should be bright, round and refractile. Hybridomas may be very sticky and require a gentle knock to the flask to detach the cells. EBV transformed cells can grow in very large clumps that are very difficult to count and the centre of the large clumps may be non-viable
2. Do not centrifuge to subculture unless the pH of the medium is acidic (phenol red = yellow) which indicates the cells have overgrown and may not recover. If this is so, centrifuge at 150 x g for 5 minutes, re-seed at a slightly higher cell density and add 10-20% of conditioned medium (supernatant) to the fresh media
3. Take a small sample (100-200µl) of the cells from the cell suspension and count the cells (Protocol 6 - Cell Quantification). Calculate cells/ml and re-seed the desired number of cells into freshly prepared flasks, without centrifugation, just by diluting the cells. Refer to the data sheet supplied with the cell line for the recommended seeding density
4. Repeat this every 2-3 days.

Key point

If the cell line is a hybridoma or another cell line that produces a substance (e.g. recombinant protein or growth factor) of interest retain the spent media for analysis.