



STERILIZATION AND FILTRATION OF MEDIA AND REAGENTS - SERUM THAWING, INACTIVATION AND TESTING

A- STERILIZATION AND FILTRATION OF MEDIA AND REAGENTS

The media (DMEM, medium 199, SGM, Nutrient mixture Ham's F14, Basal Medium Eagle) used in the cell culture laboratory are purchased as gamma ray-sterilized solutions and certified as endotoxin-free. They are purchased without L-glutamine for increased stability. L-glutamine, purchased as a sterile endotoxin-free solution, is added immediately prior to use.

The foetal bovine serum used is certified as virus (BVD-IBR-PI3) and mycoplasma-tested and, since 1988, also bovine spongiform encephalopathy (BSE)-tested. The main method for eliminating mycoplasma contamination is filtration (0.1 micrometer pore size), while gamma-irradiation is used to destroy viruses.

Human fibroblast growth factor and the epidermal growth factor are purchased as sterile filtered solutions with certified endotoxin concentration less than 0.1 ng/microg. Antibiotics (penicillin-streptomycin 10000 IU) are purchased as filtered and endotoxin-free solutions 100x. Insulin is purchased as a gamma-sterilized lyophilised powder. Before addition to the medium, 100 mg of powdered insulin is dissolved in 12 ml HCl 1N and 88 ml of distilled water; this solution is then filtered (Millipore Sterilcup, 0.22 micrometer). Aliquots of insulin solution are stored at -20°C .

Complete growth medium is prepared under the hood using disposable sterile plastic ware, and filtered using 500 ml Millipore Sterilcup (0.22 μm pore size). The cap and neck of the bottle containing the sterilized medium are protected with Parafilm and stored at 4°C .

NB: No media or reagents are sterilized by autoclaving. Most complete media are stored at 4°C for 1-2 months.



B- SERUM THAWING, INACTIVATION AND TESTING

Animal sera are the most important source of nutrients for cultured cells. They contain lipids, salts, vitamins, minerals, amino acids and other necessary components for cell growth. In order to preserve these properties for long periods of time, careful handling is crucial.

Equipment and Materials

- Laminar flow hood
- Water bath 37-56°C
- 50 ml sterile centrifuge tubes
- Foetal bovine serum

Procedures

1. Storage

Animal sera should be stored frozen at -20°C and protected from light. It is also important to avoid multiple thaw/freeze cycles because this will hasten degradation and result in formation of insoluble precipitates. To avoid this, small aliquots are prepared and immediately refrozen for future use.

2. Thawing

The serum bottle is removed from the freezer and left to stand at room temperature for 10 minutes. The bottle is then placed in a 37°C water bath, with the bottle cap clear of the water (not submerged). The bottle is shaken occasionally until the serum is completely thawed.

3. Heat inactivation of sera

This is the most commonly used procedure for destroying complement and ensuring that antibody binding will not lyse cells. The serum inactivation process consists of heating to 56°C and holding at that temperature for 30-35 minutes, with occasional gentle shaking (every 10 minutes) (Important: before heating to 56°C , the serum must be completely thawed at 37°C).



C- SERUM TESTING - CELL PLATING EFFICIENCY ASSAY

Before ordering a new serum lot, a sample of the lot should be tested on the cell lines under study. This must be done well before the old (the one in use) has run out. The testing procedure is the **cell plating efficiency assay**. The assay should employ both the new serum lot and the current serum lot, and the results compared.

Equipment and Materials

- Laminar flow hood
- CO₂ incubator
- Growth medium pre-warmed at 37°C
- Haemocytometer
- Inverted microscope
- Petri dishes 35 mm
- Aliquot of foetal bovine serum to be tested
- Aliquot of foetal bovine serum in use

Procedure

1. The cell line of interest (growing on plates or in suspension) is counted by the haemocytometer cell counting method (see Protocol "[ROUTINE CELL COUNTING AND ASSESSMENT OF VIABILITY](#)")
2. 200, 400 and 800 cells per dish are seeded, in triplicate, with 5 ml of proliferating medium onto 35 ml Petri dishes. The inverted microscope is used to check, every 2-3 days, for the presence of dark aggregates (serum sediments) in the medium.
3. At 12-14 days, the numbers of colonies are determined (when cells are seeded at low density they tend to form colonies) and the cells are examined for morphology alterations, such as formation of pseudopodia or vacuoles.
4. The number and morphology of colonies in the new serum are compared with those formed in the current serum lot. If there are no major differences, the new serum lot can be used.