Microbiological Monitoring of Hepatocyte Isolation in the GMP Laboratory

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Abstract
For clinical hepatocyte transplantation, cells need to be prepared in a sterile GMP environment. Strict regulations are in place that set the standard for this environment that cells are prepared in. These regulations control all aspects of the environment. In the United Kingdom, the laboratory must have a licence from the Human Tissue Authority to prepare cell for clinical administration. The physical parameters such as air quality, pressure, temperature and microbiology counts have to be monitored regularly usually through direct measurement. Described here are the methods for microbial monitoring of the laboratory environment and the isolated cell preparations.

Key words: microbial contamination, blood culture, environment, sterility

1. Introduction
Microbial monitoring of the laboratory should be carried out weekly. This is to ensure that any potential microbial contamination is kept within prescribed limits and that the appropriate action is taken if these limits are approached or exceeded. The room air systems must be in operation and laminar flow cabinets should be on while monitoring is taking place. Microbiological monitoring of cell preparation must be performed during every cell isolation procedure (1).

When setting up environmental monitoring of a laboratory, the number of sampling points needs to be decided to ensure adequate coverage. This will depend on the size of the room. A record sheet should be made to record results. It is also useful to make a diagram of the facility marking the position of the sampling points.
2. Materials

1. Tryptone Soya Agar (TSA) contact plates (Cherwell, Bicester, UK).
2. TSA settle plates (Cherwell).
4. BacT/ALERT bottle (BioMérieux UK Limited, Basingstoke, UK).

3. Methods

3.1. Microbial Monitoring of Laboratory

Microbiological monitoring is carried out using irradiated TSA settle plates to detect microorganisms in the air and TSA contact plates for surface contamination.

Before beginning monitoring, check the plates, do not use (a) cracked plates, (b) plates that accidentally fall open, (c) plates where the agar has been touched by fingers or the plate lid, (d) plates showing signs of microbial growth and (e) plates in which the agar has dried.

3.1.1. Settle Plate Count for Airborne Microorganisms

1. Settle plates are petri dishes containing a medium, which is usually agar-based and which will encourage and support the growth of bacteria and fungi, which land on them.
2. The purpose of the settle plate count is to monitor the cleanliness of an environment.
3. Settle plates must be inverted when being stored and incubated.
5. Label the bottom of the plate with the following information:
   a. The location code.
   b. The date.
6. Place the settle plates in the appropriate position, as indicated on the record sheet and diagram.
7. Expose the agar surface placing the lid face down next to the plate.
8. Plates should be exposed for a minimum of 1 h up to a maximum of 4 h.
9. Replace the lids and collect the settle plates.
10. Seal the lids with at least two pieces of fresh adhesive tape.
11. Plates should be placed in a bag and sealed.
12. Leave at room temperature for 3 days (to encourage any fungal colonies to grow).